



INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

I. A. R. I. 6.

MGIPC—S1—6 AR/54—7-7-54—10,000.

PLANT PHYSIOLOGY

APRIL, 1926

STUDIES ON THE OXYGEN-SUPPLYING POWER OF THE SOIL
TOGETHER WITH QUANTITATIVE OBSERVATIONS ON
THE OXYGEN-SUPPLYING POWER REQUISITE
FOR SEED GERMINATION*

LEE M. HUTCHINS

(WITH NINE FIGURES)

TABLE OF CONTENTS

I. INTRODUCTION	96
II. LITERATURE	99
III. METHOD AND APPARATUS	100
1. GENERAL CONSIDERATIONS	100
2. THE ABSORBER	101
3. THE GAS STREAM	
a. SOURCE AND INITIAL CONTROL	103
b. THE SCRUBBER	104
c. SECONDARY PRESSURE REGULATOR	108
d. THE REFINER	109
e. THE TELLTALE	111
f. THE DISTRIBUTOR	112
g. PRIMARY ORIFICE CONTROLS	113
h. THE SELECTOR	114
4. THE INDICATOR	
a. INDICATOR CELL, CHECK CELL AND GAS-ESCAPE CELL	116
b. THE COLORIMETRIC TESTS	118
c. APPARATUS FOR PREPARING AND DELIVERING THE INDICATOR SOLUTION	121
d. PREPARATION OF THE INDICATOR SOLUTION	125
e. THE COLOR STANDARDS	125
f. COLOR OF THE INDICATOR SOLUTION	126
5. ADDITIONAL NOTES ON THE METHOD AND APPARATUS	
a. CALIBRATION OF ABSORBERS AND INTERPRETATION OF INDEX VALUES	127

b. TESTING THE APPARATUS BEFORE INTRODUCING SOLUTIONS	130
c. ADJUSTMENT OF THE THROTTLE-CONTROL	131
d. USE OF THE ASPIRATOR	131
e. RUBBER-TUBING CONNECTIONS	131
6. GENERAL APPLICABILITY OF THE NEW METHOD	132
IV. EXPERIMENTATION	
1. EXPERIMENTS WITH SOILS	132
2. EXPERIMENTS WITH SEED GERMINATION	133
V. SUMMARY AND CONCLUSIONS	148
LITERATURE CITED	149

I. Introduction

The study of the oxygen-supplying power of the soil is a new one in plant physiology and ecology, although it has long been supposed that the oxygen of the soil influences plant growth. The static aspect of the soil oxygen problem has been attacked by a few investigators working experimentally and by a larger number of writers who have discussed probabilities and expressed opinions without much quantitative data. The whole subject of soil aeration, as thus far developed, is based on the same kind of conception of static conditions as has characterized almost all ecological and physiological study until very recently. Soil moisture and soluble soil salts have been much studied, but generally with the idea that the amount of water or salt actually present in each unit of soil somehow determines the growth of plants rooted therein. In the earlier literature of soil aeration this same idea has generally been followed. While the present paper was in process of preparation there appeared CLEMENTS'S (8) review of this literature, and his book has been carefully studied to determine whether there might be there recorded any discussion wherein attention had been given to the dynamic aspect of soil aeration. But no suggestion of such a method of approach appears in those pages nor is there any mention of this remarkable deficiency.

As has recently begun to be pointed out or implied by a few writers, the environmental conditions that influence plant growth and development do so through the different rates at which the environment can deliver matter or energy to the organism, or take it away. When the possible rate of delivery of any substance, for example, to the plant is higher than the rate at which absorption of that substance needs to proceed for satisfactory growth, then the plant can not suffer from lack of that substance—as far as the environment is directly concerned. Of course the plant may, under the influence of some particular environmental complex, be so constituted or in such a physiological condition as to be unable to absorb some needed substance at an adequate rate for satisfactory growth, even though the present

* Botanical contribution from the JOHNS HOPKINS UNIVERSITY, No. 79.

environmental supply be adequate; but in such cases the deficiency is not to be directly related to the environmental supply of the substance in question. It is to be related to the internal characteristics of the plant itself as influenced by the other environmental features. It is clear that an organism can not absorb any substance more rapidly than that substance is supplied from the environment to the absorbing portion of the body periphery. An organism may of course absorb a given substance at any rate that is *lower* than the maximum rate at which the environment can supply that substance. Physiology, ecology, pathology, agronomy, horticulture, and forestry, as well as all other sciences and arts that deal with life as a complex of processes need very much to consider many of their problems from this point of view.

It appears that the only influential environmental conditions thus far studied with reference to supplying power are the evaporating power of the air, that of radiation, and the water-supplying power of the soil. The first two deal with environmental rates of removal of water from the organism; these may be considered as cases where the supplying power is negative. The water-supplying power of the soil has begun to be studied (9, 15, 17, 19), but little more than the development of promising methods of thinking and experimentation has yet been achieved in that connection. It seems possible that this dynamic aspect of the soil-moisture problem may now be rapidly developed, and that the conception of environmental supplying powers in general may soon begin to attract more attention. This conception is new, however, with its terminology and all its refinements of thought still to be worked out, while its applications in forestry, agriculture, etc., are as yet undemonstrated excepting in an *a priori* way.

Generally, the logical promise of the supplying-power point of view is so great and the little study that has thus far been devoted to the water supplying power of the soil seems to be so enlightening, that it appeared desirable to make an attempt to devise methods by which the capability of the soil to supply another essential substance might be quantitatively and directly studied. This apparent desirability formed the point of departure for the studies reported in this paper.

Considering the substances that must be supplied from the environment to ordinary plants, and thinking of these with reference to the relative amount of each that is required to bring a plant to maturity, water is surely the one that should be first mentioned. As has been said, fair beginnings have been made toward the dynamic study of the environmental conditions that influence the rates of entry and exit of water from the plant. Next to water, with reference to the amounts needed by green plants, is carbon dioxide. Livingston (16) has described a small beginning of the study of

the carbon-dioxide-supplying power of the air but that of the soil has not been considered in the literature. Indeed, the literature appears to offer no suggestion that this soil feature may be important in plant physiology, ecology, etc. Oxygen, uncombined, is third in amount needed, on the list of environmental substances required by ordinary green plants, and second on the list when higher non-green plants are considered. It seems safe to suppose that plants seldom or never suffer in nature from inadequate oxygen-supplying power of the air, but it seems to be clear that differences in the oxygen-supplying power of the soil must be of great importance in determining plant growth in different soils, etc. Consideration of this soil feature lies, of course, in the field of the general problem of soil aeration. It seemed desirable to attack next the oxygen-supplying power of the soil, as a very important environmental characteristic that might be studied dynamically and quantitatively.

Another line of thought was influential in determining the point of attack for the studies here reported. If we consider the substances required by cultivated plants in the order of their possible practical control by plant growers at present, and in the order of their present importance in the selection of sites for plant growing, water is certainly first and soil oxygen is surely second. Growers everywhere can manipulate the water conditions about their plants, or select sites with regard to natural water conditions, more readily than they can similarly deal with any other feature of environmental supply. Watering, irrigation, shading, protection from wind, cultivation, soil drainage, many aspects of pruning, and the selection of plant varieties and of soils to be employed, are all clear illustrations of the practical importance of water relations in plant culture. The soil oxygen relation is also practically considered and manipulated, consciously or unconsciously, especially in cultivation, irrigation and drainage. The carbon-dioxide supply is perhaps involved in some of the usual operations of plant culture, although usually unconsciously so, and carbon dioxide may perhaps be placed as third in the present list. The carbon-dioxide-supplying power of the soil (and perhaps of the air in some cases) is somewhat readily manipulated by cultivation, drainage, irrigation, manuring, etc.

The oxygen-supplying power of the soil was made the subject of these studies because of the considerable quantities of oxygen apparently needed by ordinary plants, because many plants are thought to suffer from an inadequate supply of oxygen from their soil environment, because the soil-oxygen-supplying power may be easily altered by cultural practice, and because this soil feature may readily be consciously and quantitatively considered in selecting plant varieties and soils for cultural operations and in

modifying the operations themselves. What is first needed, apparently, is a method suitable for measuring and comparing the oxygen-supplying powers of different root environments, that is, of different soils. The dynamic water relations of plants have already been fairly well opened for further quantitative study, so that fundamental work in that field seemed to be not so pressingly needed as the general opening of the subject of the oxygen relations of roots. Furthermore, it was thought that the comparative measurement of the oxygen-supplying powers of soils seemed to offer greater practical difficulties than did the corresponding measurements of the carbon-dioxide-supplying powers of soils, and it seemed that suitable methods for the former might involve many features directly applicable to the latter. In point of fact, the method described in the following pages may, with relatively slight alterations, be adapted to the measurement of the carbon-dioxide-supplying powers of soils. Finally, growers of plants and others who deal with plants in scientific research are perhaps generally more nearly ready at present to profit by scientific advances with regard to soil oxygen than they are to make use of advances with regard to soil carbon dioxide.

These studies have to do particularly with methods for measuring and comparing the oxygen-supplying powers of different root environments, primarily soils. In a broader way, the methods here set forth may be valuable for comparative studies of soil-oxygen-supplying powers in other connections (such as that of soil micro-biology, engineering, etc.), and also for the measurement of the oxygen-supplying powers of many other kinds of environment besides soils. Thus, for example, this environmental feature may be studied for different depths in standing or flowing water, as of lakes, rivers and the ocean, for interiors of masses of culture media, etc.

The experimentation was carried out in the Laboratory of Plant Physiology of the Johns Hopkins University, under the personal direction of Professor BURTON E. LIVINGSTON, who has contributed very much to the paper as it now appears.

II. Literature

As has been said, there seems to be almost no literature at all bearing on the question of oxygen-supplying powers of soils. CLEMENTS'S (8) very thorough-going summary of the literature of soil aeration presents an apparently nearly complete review of the subject up to about 1920, but a thorough study of that monograph has not brought to light any references to oxygen-supplying power. Many writers have dealt with the different oxygen *contents* of various soils and at various depths in the soil, and the general idea of the environmental supplying power has doubtless been in

many minds. But the only statement bearing directly on this aspect of the soil oxygen question thus far encountered in the extensive literature of soil oxygen and the oxygen requirement of plants is one by CANNON (3), who says: "It is the rate of supply and not the partial pressure of the gas (in the soil air) that is important." It is of course true that the partial pressure of oxygen in a root environment may in many cases be considered as directly proportional to the environmental supplying power for that substance, but this can be regarded as true only for special cases. A soil containing but little oxygen per unit of volume might nevertheless be capable of delivering oxygen to the absorbing surfaces of roots at a considerable maintained rate, while a soil containing relatively much oxygen might soon become depleted near the absorbing surfaces and its maintained rate of delivery might consequently be low.

It will not be necessary or desirable to attempt here to summarize the literature of soil aeration further, since supplying powers have not hitherto been considered. A number of papers on the static aspect of this subject have appeared since the publication of CLEMENTS'S monograph, one of the most satisfactory of which is that of ROMELL (18). That paper is worth careful study, but it does not bear directly on the dynamic problem with which the present paper deals. A few papers that bear on the discussions of the present results will be mentioned in the following pages. A preliminary statement of the aims of these studies and of the methods used has been published by HUTCHINS and LIVINGSTON (11).

III. Method and apparatus

1. GENERAL CONSIDERATIONS

The determination of the capacity of a soil to supply elementary oxygen would require two essential units in the apparatus, an absorber and an indicator. The absorber should present to the soil a known area of absorbing surface and should be able to absorb oxygen as rapidly as the latter arrives at its surface, and the indicator should furnish means for determining the mean rate at which absorption has been occurring during a given test period. The rate at which the absorber receives oxygen would be taken as a measure of the power of the surroundings to supply that element.

The absorber here used is made by altering in a suitable manner a porous porcelain cone of the type described by LIVINGSTON (14) for use in the auto-irrigation of potted plants. The cone is embedded in the soil at any desired depth, oxygen entering by diffusion through water of imbibition held in the porous-porcelain wall, which is constantly kept wet enough to prevent any flow of gas through it. The cavity of the cone is kept practically free of oxygen by means of a stream of oxygen-free gas that carries the absorbed

oxygen from the absorber to the indicator. The gas flow is maintained constant by pressure and orifice controls, and is so regulated that it moves rapidly enough to sweep out the absorbed oxygen as it arrives in the absorber, but not too rapidly to allow the indicator to account for all of the oxygen that reaches it.

The oxygen brought to the indicator by the gas stream is absorbed by a standard quantity of a standard aqueous solution of pyrogallol and potassium hydroxide, and the rate at which absorption takes place here is taken as a measure of the rate of entrance of oxygen into the gas stream from the walls of the absorber. The rate of oxygen absorption by the indicator solution is determined colorimetrically, advantage being taken of the fact that this solution slowly becomes darker colored as its oxygen content is gradually increased. A test is begun with the color of the indicator solution exactly matching a permanent color standard and the test consists in determining the time needed for the solution color to become the same as that of a second standard darker than the first. A definite quantity of oxygen, which may be called the *colorimetric oxygen unit* for this indicator, is required to bring about this color change in a charge of indicator solution, and each test shows the number of minutes required for the absorption of one of these units of oxygen from the soil by the absorber. The several rates of absorption indicated for a series of tests are consequently considered as proportional to the reciprocals of the corresponding lengths of the test periods. Thus, if the specified color change in the indicator solution should require 15 minutes for one soil test and 29 minutes for another, the oxygen-supplying powers of the two soils would be taken as $1/15$ and $1/29$, respectively; that is, the second soil could supply oxygen only $15/29$ as rapidly as the first.

The several parts of the apparatus will be described in detail and discussed, and then a section will be devoted to additional notes on the operation of the apparatus.

2. THE ABSORBER

The absorber is a hollow cone of porous porcelain, and oxygen is absorbed by diffusion through a known area of its wet curved surface. It is prepared from a LIVINGSTON (14) porous porcelain auto-irrigator cone by grinding the surface until the thickness of the curved wall is about 0.5 mm. The grinding is done roughly at first by means of a coarse emery cloth held against the surface of the cone as the latter is rapidly revolved on a motor-driven spindle. For later stages in the grinding the cone is stationary and a motor-driven flexible shaft and sandpaper disks such as those used in dentistry are employed. As a guide to securing uniform thickness of wall,

the interior of the cone is illuminated by a small electric lamp during the finishing process. This operation is carried on in a darkened room and proceeds until the light transmitted through the porcelain wall appears uniform over the whole curved surface of the cone and is about equal in intensity to light from a similar source transmitted through ten sheets of Whatman's filter paper no. 30. The plane base of the cone is ground until it transmits light faintly, it being desirable to have the cone walls hold as small a volume of water as is practicable, in order to prevent too great a lag in the tests. The neck and the flat base, and a little of the conical surface above and below are next coated with sealing wax ("Dennison's Fine Red") to confine oxygen absorption to the thin, curved surface. The construction of the absorber now used, with its stopper and tubing connections,

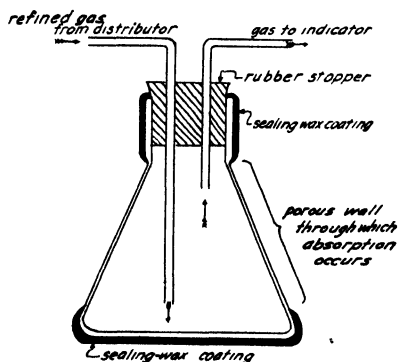


FIG. 1. Diagram showing vertical section of absorber.

is shown by diagram in fig. 1. Each absorber is given a number, and the measured area of its curved absorbing surface is recorded.

The thin, porous wall of the absorber needs to be impregnated with a substance such that the wall remains permeable to the movement of gas by diffusion but is impermeable to mass movement or streaming. Refined paraffin oil ("Nujol") was found to be well adapted for this purpose with absorbers exposed to dry environments (11, p. 135), but most soils that are moist enough to make a study of their oxygen-supplying powers of interest in physiological and ecological connections, also supply enough moisture to keep the wall of the absorber sufficiently impregnated with water (soil solution). This moisture is held in the porous porcelain by a strong imbibitional force, pressures of an atmosphere or more being required to cause gas streaming through the wall when the latter is wet. It is of course possible that the imbibed moisture may become depleted if the surrounding soil is very dry, but one of the mercury valves in the apparatus would always show clearly if the gas were to begin to stream through the wall of the

absorber. Gaseous movement between the environment and the cavity of the absorber is to take place only by diffusion through the water held in the absorber wall.

When ready for operation, the neck of the absorber is closed by a rubber stopper bearing two tubes. One tube conducts the gas stream, of oxygen-free nitrogen, into the bottom of the absorber; and the other tube conducts the gas stream, which now generally bears some oxygen, out of the top of the absorber to the indicator, where the time-period for the delivery of an indicator unit of oxygen is determined. The oxygen-supplying power of the environment being tested is calculated from the length of this time period and the area of the absorbing surface. It is expressed in terms of the quantity of oxygen delivered in a unit of time per unit of area; as, *e.g.*, milligrams of oxygen supplied per hour through a square meter of absorbing surface exposed to the soil.

3. THE GAS STREAM

(a) SOURCE OF GAS AND INITIAL CONTROL.—In the preliminary part of these studies (11) the source of the gas stream was the laboratory supply of illuminating gas, the free oxygen in the latter having been removed by alkaline-pyrogallol solution in scrubber bottles and pressure gradient for movement having been supplied by a bottle aspirator. For the later work commercial nitrogen (furnished under pressure in tanks) has been found to be superior and more convenient. The parts through which the gas passes before reaching the absorber may be considered in the order in which the gas reaches them.

Nitrogen from the supply tank passes through the partially open throttle (fig. 2, valve 1) into the chamber of the throttle control valve (fig. 2, valve 2). The gauge of this valve indicates the pressure of the gas as it leaves

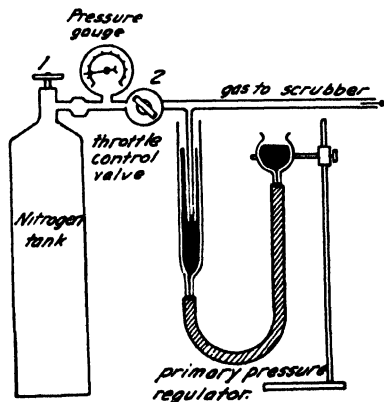


FIG. 2. Diagram showing primary control of gas-stream from nitrogen tank.

the valve. This pressure and the rate of flow are regulated by adjusting the valve and a very uniform slow rate is readily secured. The throttle itself should not be opened wider than is necessary; the pressure indicated by the gauge should be between 200 and 500 pounds. Adjustment is made by means of the throttle and the control valve from time to time as the gas pressure in the supply tank gradually decreases.

A lateral branch of the tube leading from the control valve is connected to a primary mercury pressure-regulator, shown by the diagram of figure 2. This branch extends downward into a vertical glass cylinder graduated for height (an ordinary burette is convenient). The lower, open end of the branch is closed by mercury in the cylinder and the mercury level is regulated by raising or lowering a properly supported mercury reservoir (thistle tube) connected by rubber tubing with the lower end of the cylinder. The height of the mercury column in this regulator is always such that (1) gas pressures in succeeding parts of the apparatus will never be great enough to expel stoppers or cause other damage and (2) sufficient pressure is maintained to cause continual gas-escape from the secondary pressure-regulator, which will be described later. The main tube from the throttle-control valve leads to the scrubber.

(b) THE SCRUBBER.—Commercial nitrogen contains some oxygen and the latter must of course be removed before the gas enters the absorber in the soil. This is accomplished by means of aqueous alkaline pyrogallol solution in the scrubber and in the refiner. The scrubber is composed of several like units connected in series, each unit consisting of a glass bottle of about four liters capacity, containing about a liter of scrubber solution

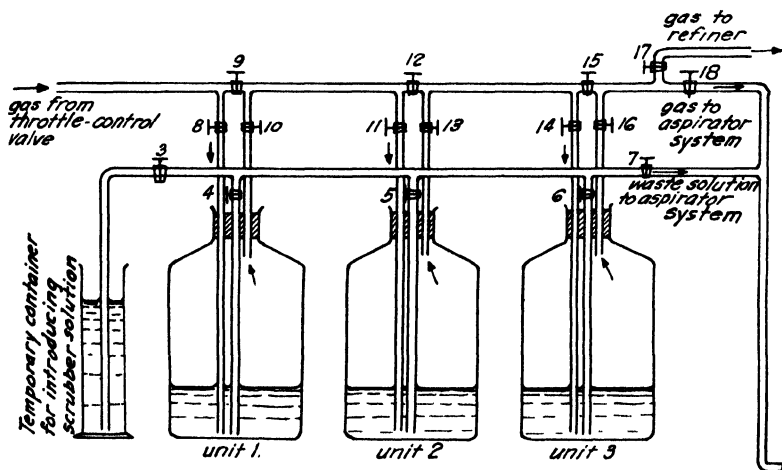


FIG. 3. Diagram showing arrangement of scrubber parts.

and closed by a rubber stopper bearing three tubes, one for introducing or removing liquid, another for entrance of gas from the throttle-control valve, and the third for exit of gas. The scrubber arrangement is shown diagrammatically in fig. 3. The gas inlet tube reaches nearly to the bottom of the bottle and the outlet tube extends only a short distance below the stopper. The outlet tube is connected to the next scrubber unit (in the case of the last unit, to the refiner). The number of scrubber units needed depends upon the amount of oxygen in the original gas supply and on the rate of flow. For commercial nitrogen used in these studies and for the continuous operation of eight absorbers four scrubber units have been found to be adequate.

Two forces operate to move the liquids and the gas, (1) the pressure of the gas as it leaves the throttle-control valve and (2) suction produced as needed by an aspirator (Chapman filter pump) operated by the laboratory water supply and connected by tubing to the various parts of the apparatus where aspiration is needed. To remove and replace the solution in a scrubber unit it is necessary that the movement of gas through that unit be temporarily stopped. The scrubber is therefore so arranged that, by closing the gas inlet and outlet cocks of any unit and opening a by-pass, the unit in question may be disconnected from the system without interrupting the gas stream as a whole. The operation of removing and introducing scrubber solution is accomplished without admitting any air to the unit, and the latter is then again connected with the system.

The scrubber solution is prepared within each unit by mixing an aqueous solution of pyrogallol and one of sodium hydroxide. About 250 cc. of a 15-molecular solution of the hydroxide is passed into the unit and this is followed by about 750 cc. of a 4-molecular solution of pyrogallol. [See ANDERSON (1)]. For the present purpose the solution described in the text has been found to be more satisfactory than the one recommended by ANDERSON. The hydroxide solution is prepared by dissolving in distilled water the entire contents of an original 1-lb. package of sodium hydroxide to make 600 cc. of solution. For the pyrogallol solution 500 grams of the crystals is dissolved in distilled water to form a liter of solution.

To introduce one of these solutions, or water for cleansing, into a scrubber unit, the liquid to be introduced is first placed in a scrubber-supply container (fig. 3) graduated so that a known amount can be drawn into the liquid conduit, which is extended for this purpose. Starting with the units empty and all cocks of the scrubber-system closed, aspiration is applied to all the units by opening the cocks in the gas outlet tubes and the by-passes. After air has been removed as far as possible, and while the aspirator is still functioning, the first cock in the liquid conduit (cock 3) is opened, as is

also the cock in the branch of that conduit (cock 4) leading into the first scrubber unit. The solution from the container now begins to flow into the first unit and the flow is stopped by closing cock 4 when the desired amount (250 cc.) has been transferred. Similarly, the cocks in the branches of the liquid conduit leading to succeeding units are successively opened to allow introduction of the hydroxide solution and are closed after the proper amount has entered. The excess of hydroxide solution left in the graduated container is now replaced by pyrogallol solution, the container being first thoroughly rinsed, and the required amount (750 cc.) of the latter is passed into each unit by the method described for the introduction of hydroxide solution. The conduit for liquids and its branches are now washed with distilled water. Thorough mixing of the two solutions may be hastened by gently agitating each scrubber bottle. All open cocks of the scrubber system are to be closed. Each unit now contains the proper amount of fresh solution but, because of the continued action of the aspirator during the introduction of the solutions, their internal gas pressure is likely to be far below that of the external atmosphere. To relieve the partial vacuum, nitrogen is next introduced into the units from the throttle-control valve in the following manner: The throttle-control valve is slowly opened until gas escapes freely but not violently from the primary pressure-regulator. The first cock of the gas conduit (cock 2) is opened and then the cock of the gas inlet to the first scrubber unit (cock 8) is opened *cautiously*, care being taken that mercury or air is not drawn in from the regulator. When the gas pressure in the first unit has become slightly greater than that balanced by the mercury column in the regulator, as is indicated by gas escaping from the latter, the cock controlling the gas outlet from that unit is opened. Then the cock controlling the gas inlet for the second unit is *cautiously* opened and this unit is supplied with nitrogen. The same procedure is followed for this and succeeding units as has been described for the first unit. After all units have been cared for the cock in the gas conduit leading from the scrubber (cock 17) is opened, allowing the gas to pass on to the refiner.

Since the gas from the supply tank passes through the scrubber units in series, the power of the scrubber solution to absorb oxygen decreases more rapidly in the first unit than in succeeding ones, and the solution in the first may have lost most of its absorbing power while that in the other units still remains very potent. To judge of the condition of the scrubber solution in any unit, the gas connections of that unit are closed and a small amount of the solution is passed, by suction from the aspirator, up into the conduit for liquids, which is a glass tube of about 5 mm. bore, and is viewed in ordinary light. If the sample appears opaque the oxygen-absorbing

power of the solution in that unit may be considered as low and a renewal of solution is desirable. In each unit the vertical branch of the conduit for liquids is usually filled with water, as is also the conduit itself, and to bring the sample into the conduit for observation it is necessary to allow the contents of the vertical branch to flow into the conduit first, letting it be followed by the sample. If this test indicates that a change of solution is not yet necessary the solution should of course be removed from the conduit and the latter filled with water drawn from the scrubber-supply container. Finally, the gas connections are opened, placing the tested unit again in operation.

If the sample drawn up into the liquid conduit appears opaque, the unit in question should be emptied, the waste solution being discarded through the aspirator system. After the solution has just been removed the gas pressure in the unit is low, as it was before the unit was originally charged. Aspiration is now cut off (cock 7) and the unit is recharged by transferring into it the solution from the next succeeding unit, the gas connections of which must first be closed, assuming that that solution is still potent. The unit thus emptied is first partially exhausted by means of the aspirator system operating on the liquid conduit, aspiration is again cut off (cock 7) and the unit is recharged as before from the next succeeding unit. Finally the last unit is recharged with new solution, introduced from the supply container in the manner described for originally charging the scrubber units. By this method of procedure the most potent oxygen-absorbing solutions are always in those units nearest to the refiner, to which the gas passes after leaving the scrubber. After all emptied units have been recharged the gas connections are once more opened and the entire scrubber series goes again into operation.

To illustrate a method of transferring scrubber solutions, let it be assumed that the solution in the first unit is to be discarded, that the solutions in the second and third units are to be transferred to the first and second units, respectively, and that fresh solution is to be prepared in the third unit. First, all open cocks of the scrubber system are to be closed. Aspiration is applied to the conduit for liquids and then the cocks that control the liquid conduit between the first unit and the aspirator system are opened (cocks 7 and 4). Solution passes from the first unit into the conduit for liquids and is discarded through the aspirator system. Aspiration is continued until all of the liquid and as much gas as possible has been removed from the first unit. The exhaust is now cut off from the conduit for liquids by closing cock 7. To pass the solution from the second unit into the first, the cock controlling the branch of the conduit for liquids leading into the second unit (cock 5) is opened, permitting the gas pressure above the solu-

tion in that unit to force the solution over into the first unit. The gas pressures in the two units are allowed to become adjusted, then the cock controlling the branch of the conduit for liquids leading into the first unit (cock 4) is closed and the cock controlling the branch of the conduit for liquids leading into the third unit (cock 6) is opened, allowing the solution to pass from the third into the second unit. Again gas is allowed to follow the liquid until the pressures in the two units are adjusted. Fresh scrubber solution is now introduced into the third unit, in the manner already described. Then the conduit for liquids and its branches are rinsed with water and the gas from the throttle-control valve is allowed to adjust the pressure in all of the units. The cock in the gas conduit leading from the scrubber (cock 17) is opened, permitting the gas to pass on to the refiner. Should it happen that only a part of the solution from one unit has passed into the preceding unit of the scrubber series when the gas pressures in the two units become equalized and the solution ceases to flow, as is particularly apt to be the case in transferring solution from the last unit of a scrubber, the gas in the unit receiving the liquid is rarefied by opening the necessary cocks and applying the exhaust through the gas conduit, the by-passes and the gas outlet tube of the partially charged unit, until all of the solution has been transferred.

Ordinarily one charge of solution in the units should suffice to remove the oxygen from the gas in one nitrogen tank of the size and compression used in this work, and it should not usually be necessary to test the scrubber solution excepting when a new cylinder is introduced.

Cylinders of nitrogen received from the factory are each calculated to contain a quantity of nitrogen such that, if allowed to expand under atmospheric pressure, the gas would occupy a volume of 100 cu. ft. If the requisite rate of gas flow is taken as 10 cc. per minute, which is greater than the actual requirement in the studies here reported, a single cylinder of gas should last about 185 days, with eight absorbers in continuous operation. Commercial nitrogen may contain as much as 1 per cent. of oxygen, and it may consequently be roughly estimated that the scrubber should be capable of removing oxygen from the gas stream at a continuous rate of 0.01 cc. (atmospheric pressure) per minute. With several scrubber units in series a considerable reserve of scrubbed gas is generally available and it is consequently possible to pass rapidly a liter or more of oxygen-free gas into other parts of the apparatus whenever that may be necessary.

(c) SECONDARY PRESSURE REGULATOR.—The secondary pressure-regulator is inserted in the main gas conduit between the scrubber and the refiner. Its arrangement is shown by the diagram of fig. 4. It is similar to the primary pressure-regulator. An open branch of the conduit is ex-

tended into a vessel of mercury, the end of the tube reaching to a depth so that the mercury pressure will correspond to a pressure on the gas at this point slightly greater than that necessary to maintain gas flow. Gas is always slowly escaping from this regulator, more rapidly when for any reason the pressure of the incoming gas becomes a little too high. Only a small amount of gas is thus wasted, however, and the regulator makes it certain that the gas pressure from this point forward in the gas stream can never be higher than that for which the regulator is adjusted.

Coming from the scrubber, the scrubbed gas passes the secondary pressure-regulator and proceeds to the refiner, which removes any traces of oxygen that may have escaped the action of the scrubber.

(d) THE REFINER.—The refiner consists simply of a series of three Pettenkoffer tubes of aqueous alkaline pyrogallol solution so placed that the gas bubbles traverse about two meters of liquid in passing through the three tubes. By means of gas supply from the scrubber, liquid supply from the mixer (to be described below) and a waste conduit of the aspirator system, the solution, and water for cleansing, may be passed into or discarded from the refiner tubes individually with only brief interruption of the gas stream. The refiner and its connections are shown by the diagram

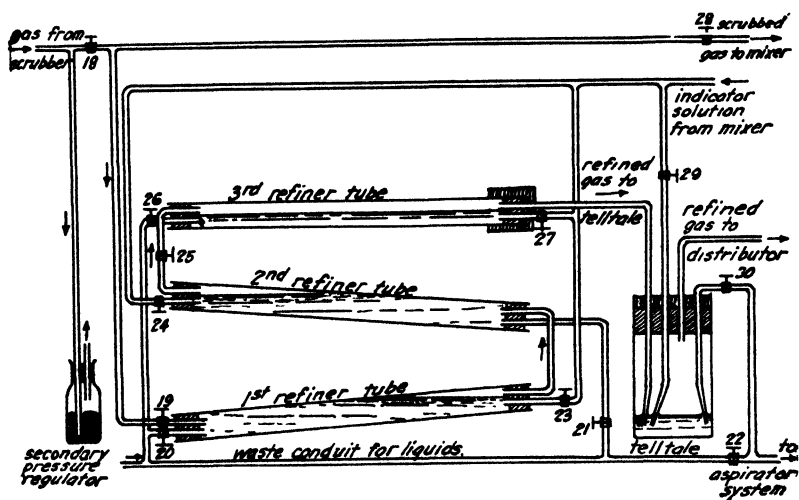


FIG. 4. Diagram showing arrangements of secondary pressure-regulator, refiner and telltale.

of fig. 4. As in the case of the scrubber, the cocks for controlling the liquid conduits consist of Hoffman tubing clamps on heavy-walled rubber tubing, but glass stop-cocks are used to control the gas conduits of the refiner sys-

tem and of all succeeding parts of the gas system. Furthermore, each connection between parts of the gas conduit from the refiner forward is adequately protected against gas diffusion by a mercury seal or by a glass sleeve poured full of sealing wax or paraffin.

The alkaline pyrogallol solution for the refiner is somewhat different from that used in the scrubber, being prepared with potassium hydroxide instead of sodium hydroxide and being more dilute than the scrubber solution, with more sensitive color response when oxygen is absorbed. It is prepared in the mixer in the same general way as is the indicator solution itself. A refiner solution composed of one part of the stock potassium hydroxide solution and two parts of the stock pyrogallol solution is satisfactory. The preparation and use of these stock solutions is dealt with later, where the indicator solution is described.

The refiner is put into operation, starting with all its cocks closed and its tubes containing only air, in the following manner. The cocks are opened that control (1) the gas conduit between the second and third refiner tubes (cock 25), and (2) the waste conduit leading from the telltale bottle (cock 30), which is the next unit in the series (see sec. e). Next the cocks nos. 23, 24, and 27 controlling the liquid inlets are opened and allowed to remain open long enough to permit solution from the mixer to force the air out of the conduits for liquids. The cock (no. 19) controlling the gas inlet to the refiner is now partially opened, allowing gas from the scrubber to enter, thus sweeping the air in the refiner tubes out through the waste conduit of the telltale bottle. When a volume of nitrogen has been passed into the tubes somewhat greater than the capacity of the latter, under atmospheric pressure, the cock (no. 19) controlling the gas inlet of the refiner is closed and the cock (no. 23) controlling the liquid conduit leading to the first refiner tube is opened and remains open until the refiner tube is nearly filled with solution. Of course the solution meniscus must not be allowed to reach the gas outlet. As liquid enters the tube it replaces the gas, which escapes through the gas conduit and the telltale bottle. Similarly, solution is introduced successively into the second and third refiner tubes, using the proper cocks (nos. 24 and 27). When all three refiner tubes are ready for operation, the cock (no. 19) controlling the gas inlet to the first refiner tube is opened, it being first ascertained that the gas pressure in the conduit is correct, as shown by the bubbling of gas out of the secondary pressure regulator. Gas bubbles move through the refiner tubes and the refined gas escapes through the telltale bottle. If the refiner solution is much discolored it is allowed to remain in the tubes about 20 minutes to permit absorption of residual oxygen left in the tubes at the time of filling, after which it is discarded and the tubes are refilled with fresh solution from the mixer.

The method of discarding and introducing solution after the refiner is ready for operation (or has been operating) is as follows: All open cocks (nos. 19 to 27, inclusive) of the refiner are to be closed. The two cocks (nos. 20 and 19, respectively) that control the waste conduit from the first tube and the gas inlet to this tube are opened. Gas enters the first tube from the scrubber and forces the liquid out through the waste conduit and aspirator. The cock (no. 19) controlling the gas inlet to this tube is then closed and the cock (no. 23) controlling the liquid inlet is opened to permit the desired amount of solution to enter, after which the latter cock is again closed. To remove the solution in the second refiner tube, the cock (no. 21) controlling the waste conduit from this tube is opened, as is also the cock (no. 19) controlling the gas inlet to the whole refiner. Gas now bubbles through the first refiner tube, enters the second and there displaces the solution as the latter passes into the waste conduit. To introduce fresh solution into this tube the two cocks (nos. 25 and 24, respectively) that control its gas outlet and solution inlet are opened, to allow the desired amount of solution to enter from the mixer, after which the liquid inlet cock (no. 24) is again closed. Gas from the second refiner tube now passes into the third. To discard the solution from that tube, the cock (no. 26) controlling its waste conduit is opened. Gas now bubbles through the first and second tubes into the third, forcing the liquid out through the waste conduit. The cock (no. 26) controlling this conduit is now closed, as is also the cock (no. 25) controlling the gas inlet to the third refiner tube, and the cock (no. 27) controlling the liquid inlet is opened and remains so until the desired amount of solution has entered the tube, forcing the gas out through the telltale bottle. The entire refiner is now put into operation by cautiously opening the cock (no. 25) that controls the gas conduit between the second and third refiner tubes. Gas from the scrubber should now bubble through the three refiner tubes in series and then pass on to the telltale bottle.

(e) THE TELLTALE.—The telltale (fig. 4) furnishes a continuous test for the presence of oxygen in the gas after the latter has passed through the refiner and is about to be distributed to the absorbers. The telltale is a rubber-stoppered bottle with a sealing-wax seal and with four tubes leading into it as follows: (1) for the entrance of gas from the refiner; (2) the exit of gas to the distributor; (3) for the introduction of solution from the mixer; and (4) discharge of solution through the aspirator system. Gas from the refiner enters the telltale bottle at a point near the bottom of the latter, bubbles through a layer of solution about a centimeter deep and leaves near the top of the bottle. The solution used here is the same as that used in the indicator, to be described later.

Throughout all tests the solution in the telltale bottle should remain without appreciable change in color. Any discoloration of the solution indicates that some oxygen is passing the refiner and if this ever occurs, the refiner, the scrubber, or the gas connections require attention. Whenever this solution has darkened (it may acquire a light orange-brown color after several days) it should be renewed with fresh indicator solution from the mixer, and it is generally well to make this renewal at least every five days.

To place the telltale bottle in operation, beginning when it contains only ordinary air, refined nitrogen is first passed through it for about 15 minutes, the air being thus swept out and discharged through the aspirator system. Indicator solution is then introduced by opening the cock (no. 29) in the solution inlet, which is afterwards again closed.

When the telltale bottle has been in operation and its solution is to be removed, the two cocks (nos. 29 and 30, respectively) in the solution inlet and solution outlet are opened. Fresh solution from the mixer flows into the bottle while solution is at the same time flowing out, and both of these cocks remain open until the solution in the bottle shows the same color as the solution entering from the mixer, then both these cocks are again closed. During the process of renewal, the cocks are to be adjusted so as to maintain the solution level in the bottle approximately constant. The gas flow through the telltale continues uninterrupted while the solution is being renewed. The refined nitrogen flows through the telltale to the distributor, on its way to the absorbers.

(f) THE DISTRIBUTOR.—The distributor consists of a number of units, one for each branch that leads from the main gas supply, from the telltale, to an absorber. Each unit consists of two mercury-valve devices, one allowing the branch to be opened or closed by a simple operation, the other eliminating the possibility of any backward movement of gas from the absorber. The arrangements of the distributor are shown diagrammatically in fig. 5. The device for starting and stopping the gas flow consists of a vertical hollow cylinder of "Pyrex" glass, measuring 12 cm. in length and

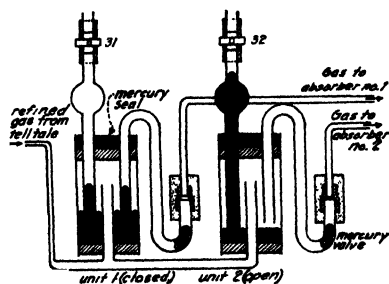


FIG. 5. Diagram showing arrangement of distributor parts.

3 cm. in diameter, closed at each end by a rubber stopper, the upper stopper being inserted far enough to allow for a mercury seal above. A branch tube from the main gas supply, leading from the telltale, perforates the lower stopper and extends upward into the cylinder nearly to the upper stopper. The upper stopper bears an S-shaped glass tube, the inside diameter of which is at least 7 mm. One end of this tube reaches down into the cylinder nearly to the lower stopper, while the other end is vertical for a distance of several centimeters and is finally connected directly with the absorber. The upper stopper also bears a straight vertical glass tube which extends downward into the cylinder to within about 3 mm. of the lower stopper. This tube is expanded into a small bulb above the mercury seal at the upper end of the cylinder, and its upper end is closed by a piece of rubber tubing bearing a Hoffman clamp. In the cylinder is a layer of mercury with its upper surface four or five mm. above the end of the S-tube, which it closes.

The device for preventing reflux of gas from the absorber consists of a plug of cotton supporting a mercury plug about 5 mm. high placed in the final vertical arm of the S-tube just below its union with the tube that leads to the absorber.

To open the unit and allow gas to flow from the telltale to the absorber, suction is applied to remove mercury from the cylinder by transfer to the vertical tube and its bulb, so that the mercury surface in the cylinder falls below the end of the S-tube, thus opening the latter and allowing gas to flow from the gas conduit through the cylinder chamber and the S-tube, and so on to the absorber. The clamp above the bulb is closed and the mercury remains in this position. The mercury resting on the cotton plug at the outer end of the S-tube is agitated as the gas bubbles pass around it and serves as a valuable indicator to show whether the gas is flowing properly. To close the unit, the clamp is opened and the elevated mercury returns to the cylinder, closing the S-tube.

Refined gas from the telltale passes through each open unit of the distributor to the corresponding absorber, from which it moves, through an orifice control, to the selector. By means of the latter it is directed either to the outside, being thus allowed to escape into the atmosphere, or to the indicator where the colorimetric tests are carried out.

(g) THE PRIMARY ORIFICE CONTROLS.—Into the gas line leading from each absorber is introduced an orifice control, a device for controlling the rate of gas movement. This consists of a glass tube about 4 cm. long and 3 mm. bore, containing a plug of firmly packed, dry kaolin, guarded on each side by a plug of cotton. The device is shown in the diagram of fig. 6. These primary controls are inserted in their respective gas line by ordinary rubber-tubing connections thoroughly bound-on, and the whole con-

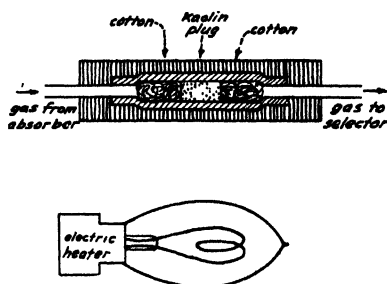


FIG. 6. Diagram showing orifice control.

nection is in each case surrounded by a glass sleeve poured full of sealing-wax. Before each control is inserted its kaolin plug is tested and adjusted as to length so that, with the gas pressure dealt with, the desired rate of gas movement is secured. The kaolin plug should be packed very firmly in the tube and adjustment is made by adding or removing kaolin so as to lengthen or shorten the plug. With the apparatus used in these studies each plug was adjusted so as to allow about 0.8 cc. of gas to pass through it per minute.

Lest the kaolin might sometimes be cool enough to collect liquid water, by condensation, from the passing gas, it is well to maintain the temperature of all the orifice control tubes somewhat above the maximum temperature expected in the absorbers and in the gas tubes leading from them to their primary orifice controls. This is conveniently done by allowing the control tubes to lie side by side across the top of a small wooden chamber in which is kept in constant operation a small electric lamp, the tubes being protected from too intense heat by means of paper lying just beneath them and above the lamp.

Besides the primary orifice controls for the gas streams from the several absorbers, there is also included in this arrangement a similar orifice control for a stream of purified nitrogen, led from the refiner directly to the selector.

(h) THE SELECTOR.—The gas from any absorber, or from the refiner, after passing through the corresponding primary orifice control, may be directed into the indicator cell by means of the selector or it may be released into the atmosphere. The selector is an arrangement by which the indicator may be effectually connected or disconnected at will with any one of the nine tubes that lead gas to this point in the apparatus. Several absorbers are generally in operation at the same time, the gas passing through each one being conducted by its own tube to the selector. Ordinarily the gas streams from all absorbers empty continuously into the air at the selector. Any one stream may be selected here and directed through

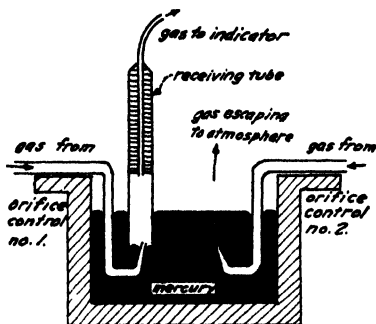


FIG. 7. Diagram showing arrangement of selector parts.

the indicator while the rate of oxygen delivery from the corresponding absorber is being determined.

The arrangement of the selector is shown in the diagram of fig. 7. The device consists of (1) a cylindrical glass vessel about 8 cm. in diameter and 4 cm. deep, containing mercury to a depth of 2 cm., (2) a series of glass escape-tubes, one for each primary orifice control, (including the control for purified nitrogen from the refiner as well as those for gas from the several absorbers) each of which penetrates into the mercury near the edge of the vessel and terminates with an upwardly directed orifice 1 mm. in diameter about 5 mm. below the free mercury surface, (3) a vertical glass receiving-tube about 6 mm. in diameter with its lower end dipping into the mercury, this tube being continued by means of a lead tube of about 1 mm. bore that leads directly to the gas entrance of the indicator cell. The lead tube is about 30 cm. long and its flexibility allows the glass receiving-tube to be moved about and placed over one or another of the gas orifices without lifting its lower end from the mercury. A plug of dry cotton guards the opening from the receiving-tube into the lead tube. Ordinarily the receiving-tube rests over the orifice for purified nitrogen and a stream of this gas flows continuously through the indicator. When the rate of oxygen supply from a given absorber is to be determined, the receiving-tube is lifted slightly, moved laterally through the mercury and lowered over the orifice for the absorber that is to be tested. When a determination is complete the receiving-tube is shifted to the orifice for another absorber or is returned to the orifice for purified nitrogen.

To guarantee an efficient mercury seal about the vertical glass receiving-tube of the selector, care must be taken that *clean* mercury is always in contact with *clean* glass. The mercury-air surface of the selector should not be allowed to become dusty or to accumulate a film that may adhere to the glass wall of the receiving-tube as it is lifted and moved through the mercury; for even with a mercury seal a centimeter in height, if the con-

tacts are not clean, oxygen may diffuse along the glass wall of the receiving-tube and enter the gas stream at a rate sufficiently rapid to give a test-time of a few hours. Whenever the blank nitrogen-check test produces any color change in the indicator solution and the telltale indicates during the same period that the gas stream as it enters the distributor is free from oxygen, attention should be given to the selector before suspecting leaks elsewhere. A guard of cardboard is usually kept over the well of the selector and this serves to protect the mercury surface from dust and to prevent droplets of mercury from being thrown from the selector by the bursting gas bubbles from absorbers that are not being tested.

Actual entrance of oxygen into the gas stream through a leak in the mercury seal of the selector is not likely to occur except after long periods of continuous operation and this difficulty should be entirely avoided by frequently cleaning the glass receiving-tube and the mercury-air surface, as with a piece of moist chamois skin. Occasionally it may be necessary to remove the mercury from the container and to thoroughly clean the mercury, the container, the glass inlet tubes and the glass receiving-tube.

The ordinary laboratory method of cleaning mercury by passing it through a pin-hole in paper held in a funnel has been found satisfactory in these studies, providing the mercury does not contain considerable amounts of amalgam; washing by dilute nitric acid is rarely necessary.

4. THE INDICATOR

(a) INDICATOR CELL, CHECK CELL AND GAS-ESCAPE CELL.—Determination of the rate of delivery of oxygen in the gas stream from an absorber is made in the indicator cell. The indicator system of which this cell is a

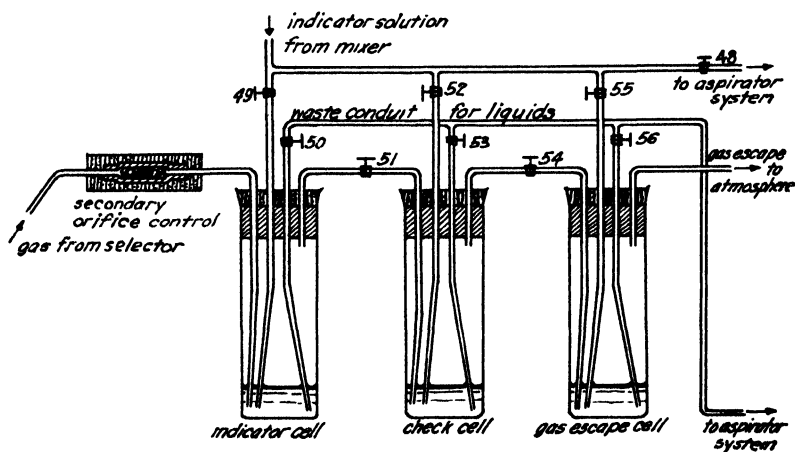


FIG. 8. Diagram showing arrangement of indicator parts.

part is shown by diagram in fig. 8. The system consists of a secondary orifice control similar to the ones already described, but with somewhat less kaolin resistance, and three vial cells, namely, the indicator cell, the check cell, and the gas-escape cell. The three cells are so constructed and connected that a measured quantity of indicator solution may be introduced from the mixer into any cell and subsequently discarded through the waste conduit for liquid. Gas from the selector passes through the secondary orifice control and then through the three cells in the order named, finally escaping into the atmosphere from the gas-escape cell. The three cells are alike in construction. Each consists of a flat-bottomed, cylindrical glass vial about 2.2 cm. in diameter and 6.5 cm. high, closed at the top by a hermetically sealed rubber stopper traversed by four tubes. One tube is for gas entrance and extends to the bottom of the cell, another is for gas exit and terminates just below the stopper. One of the remaining tubes is for the introduction of indicator solution into the cell, and the other is for the removal of solution, both of these extending to the bottom of the cell. The three tubes that reach to the bottom are somewhat bent so that their orifices lie near the lateral wall, thus leaving the mass of solution as it stands in the cell free from obstruction excepting near the right and left sides. The gas outlet from the first cell is connected to the gas inlet to the second and that from the second is connected to the inlet to the third. Each of these connections has a stopcock (nos. 51 and 54, respectively). The vial of the indicator cell has an India-inked file mark on the front and another on the back side, so placed that the center of the meniscus of 1.4 cc. of solution, as it stands in the cell, lies in the plane of the two marks. These calibration marks make it possible to operate the cell always with the same volume of solution. The measurement of the amount of solution placed in the cell might be more precise, but the precision obtained in the way here indicated has been found to be sufficiently great for the work in hand. The check cell and the gas-escape cell require no calibration.

Air is first swept from the three cells by passing a stream of purified nitrogen through them from the selector, cocks 51 and 54 being open. When the originally contained air has been displaced by nitrogen, indicator solution is introduced from the mixer. To introduce a charge of solution into the indicator cell, cocks 37 and 49 are opened, cocks 48, 52, and 55 being closed. The gas stream is allowed to continue during the operation. Similarly, solution is introduced into the check cell by opening cock 52, cocks 49, 48 and 55 being closed, and into the gas-escape cell by opening cock 55, cocks 49, 52 and 48 being closed. About a cubic centimeter of solution is used in each of the last two cells, but measurement is unnecessary.

In operation, the solution in the indicator cell darkens and must be renewed with each test. The solution in the check cell and that in the gas-escape cell should not darken at all during a test. Before the beginning of each test the operator should be sure that the indicator solution in the check cell is free from discoloration. The solution in the gas-escape cell may darken during periods when the gas stream is not flowing through the series, but its color is not important. Any slight darkening of the solution in the check cell must be detected immediately, however, for such color changes would indicate that some oxygen is passing the indicator cell. This might occur if the rate of oxygen supply in the gas stream from the selector were too great to be determined. In the studies here reported the gas always flowed from the absorber through the selector and indicator at a rate of about 0.8 cc. per minute and the arrangement here described always gave complete oxygen absorption in the indicator cell (without any color change of the solution in the check). If the oxygen content of the entering gas is too high for this arrangement, no test can be made.

(b) THE COLORIMETRIC TESTS.—Any oxygen contained in the gas stream from the absorber is taken up by the solution in the indicator cell, which gradually changes color, becoming darker as the test proceeds. The rate of this color change is used as a measure of the rate at which the gas stream delivers oxygen to the indicator cell, which is taken as equivalent to the rate of entrance of oxygen into the absorber itself.

To determine the rate of change of color in the indicator solution, two standard-color solutions are employed. Color solution A is pale and matches the color of the indicator solution when a very small amount of oxygen has been absorbed. Color solution B is considerably darker than A and matches the color of the indicator solution when a larger amount of oxygen has been absorbed. These solutions are housed in sealed vials, similar in size and shape to the indicator cell, two such vials being used for each solution. When the color of the indicator solution is being compared with that of standard A, the two vials containing this standard are placed to the right and left of the indicator cell, on the same level with it and as close to it as is convenient. When comparison is being subsequently made with standard B, the vials of standard A are replaced by those of B. Color comparison of the solution in the indicator cell with that of a color standard is made by visual observation with transmitted light from an incandescent electric lamp, the latter enclosed in such a way that all three vials are equally illuminated from behind and are viewed from the front. Equal illumination of the three vials is secured by means of a vertical white-paper diffusion screen between them and the lamp. In front of the vials is a vertical black cardboard guard with a rectangular window about 4 mm. long and 3 mm. high, opposite the middle portion of the mass of solution in each

vial. The middle window is so placed that the observer's eye receives through it only a rectangular beam of light transmitted horizontally through the unobstructed solution in the indicator cell, and the two lateral windows are on the same level, each being about 2 cm. from the middle one. From each lateral window the eye receives a beam of light like that received from the middle window but transmitted through a color standard.

The arrangement of the color standards may be modified by placing standard A to the left and standard B to the right. The former method is the more precise, but the latter method is convenient and is usually employed with test times of 15 minutes or less, it being particularly useful with test times of about four minutes, as in standardizing absorbers.

The source of light used was a "Mazda" lamp of 60 watts capacity, operated with an alternating current of 110 volts, and the standard-color solutions used are based on the characteristics of the radiation emitted by a seasoned lamp of the above-named brand and capacity. If light of other qualities or intensities were used one or both of the standard-color solutions would doubtless need to be modified.

The rate of color change in the solution in the indicator cell is measured as the length of time required for that solution to change from a color that matches standard A to one that matches standard B. In preparing for a test, the gas from the absorber to be studied is first allowed to flow through the indicator for at least 15 minutes, after which the solution in the indicator cell is replaced by a fresh charge from the mixer. The gas stream continues and the observer records the time at which the color of the solution in the indicator cell matches that of the pale standard. Observations are then made at suitably frequent intervals until the solution in the indicator cell is judged to have acquired the same color as that of the darker standard, when the time is again recorded. The length of period required for this definite amount of color change is thus determined. It may be called the *test time*. Obviously, the length of the test time may be considered as reciprocally proportional to the mean rate at which oxygen entered the absorber during the test. Thus, if the test times for two tests on the same absorber differently exposed to oxygen supply were 20 and 30 minutes, the rate of oxygen supply in the second case would be two-thirds as great as in the first.

Without further considerations of standardization, the method as thus far described suffices for comparing the oxygen-supplying powers of different exposures of the same absorber, provided that the absorber is capable of delivering oxygen to the gas stream as rapidly as oxygen is delivered by the surroundings to the external absorbing surface of the absorber, and also provided that the rate of delivery of oxygen through the absorber wall

to the gas stream is not so great as to produce color change in the check cell or to give a test time too short for satisfactory determination. When the test time is less than 15 minutes the indicator cell should be agitated at every observation and for very short times it should be continually agitated throughout the test. For such short test times, even with continuous agitation of the indicator cell throughout the test, the color change of the indicator solution is apt to be more or less premature or prepunctual. No difficulty has been experienced with the determination of the beginning of the test-time period, but the determination of its end requires special care when the test time is less than 20 minutes. In such cases the color of the indicator solution may be observed to match that of color standard A and, after the gas flow has been stopped, the color change may be reversed and the indicator solution may become somewhat paler. This indicates that the test time recorded is shorter than it should be. It is therefore desirable to bear in mind this prepunctuality of the color change when test times of 15 or 20 minutes, or less, are encountered. When the color end-point is reached the gas flow is stopped and agitation of the indicator cell is continued for a few minutes. If the indicator solution becomes paler the gas flow is resumed for a minute and the treatment is repeated, till the indicator solution maintains its agreement in color with standard B for five minutes after the gas flow has ceased. The exact explanation of the phenomenon here called prepunctuality has not been worked out. Perhaps the method may be improved in some way so as to avoid this phenomenon altogether. At worst, however, it makes necessary a little special care with test times that approach the limit of the method. It really introduces no serious difficulty in the actual use of the method, for the test times for different absorber exposures at the same depth and in the same soil mass are apt to vary considerably, because of the group of unknown reasons usually called chance. It seems safe to say that observed test times of 15 minutes or less are, even without special precautions, not to be corrected by increments of more than 7 or 8 minutes. Thus, an observed test time might be 15 minutes and the requisite correction might increase this to less than 25 minutes. A correction graph may easily be worked out, but such refinement is hardly needed in the present stage of the study of environmental oxygen-supplying power. Any test time less than an hour or two is to be considered simply as short, indicating high oxygen supplying powers of the environment in question.

To renew the solution in the indicator cell the used solution is discarded and a fresh charge is introduced from the mixer at the same time. The mixer connections are shown by the diagram of fig. 9. Cock 51 (fig. 8) is closed and cocks 49 and 50 are partially opened simultaneously. The ad-

justment of these cocks is so accomplished that the level of the solution meniscus in the indicator cell is kept practically constant during the operation. As fresh solution enters, the used solution is swept out through the waste conduit. When the solution in the indicator cell appears to be definitely paler than color-standard A, cocks 49 to 50 are closed and cock 51 is opened. The solution is then brought to the proper level by slightly opening cock 49, if more solution is to be added, or by slightly opening cock 51 if some solution is to be withdrawn, the proper level being determined by means of the two marks on the indicator vial as mentioned previously.

Whenever the indicator solution has been allowed to become much darker than the darker color standard, and tests are to be resumed, the indicator cell is recharged twice in immediate succession. The first new charge is allowed to stand for a period of about ten minutes, with the gas stream to be tested continuously directed through the cell. The cell is then recharged again and tests may be resumed.

(c) APPARATUS FOR PREPARING AND DELIVERING THE INDICATOR SOLUTION.—The indicator solution is an aqueous solution of pyrogallol and potassium hydroxide, the volume-molecular concentration of the former being 0.15 and that of the latter 2.0. A stock solution of each is first prepared and then proper amounts of these are brought together under nitrogen in the mixer. (The arrangements of the mixer and related parts of the apparatus are shown diagrammatically in fig. 9). The mixture is then properly diluted by the introduction of de-oxygenated distilled water. The mixer is connected with the liquid conduit that supplies the indicator cell, the check cell, the gas-escape cell and the telltale, so that indicator solution may be supplied to each of these separately.

With reference to fig. 9, the reservoir for oxygen-free distilled water is connected (1) with a burette for measuring water to be passed into the mixer, (2) with another burette for measuring water to be passed into the receptacle for the preparation of stock pyrogallol solution, (3) with the distilled water supply, (4) with the de-oxygenated illuminating gas supply and (5) with the aspirator system. The several burettes connected with the reservoir are filled from it by gravity. Water is drawn into the reservoir, from the distilled water supply, through cock 39, suction being applied from the aspirator system through cock 46, while all other cocks (34, 44 and 40) connected with the water reservoir are closed. When the desired amount of water has passed into the reservoir, the water supply is cut off (cock 39), and the water in the reservoir is next practically freed from oxygen by permitting de-oxygenated illuminating gas (from cock 40) to bubble through it and pass out through the aspirator system. De-oxygenated nitrogen might have been employed but illuminating gas was used in this

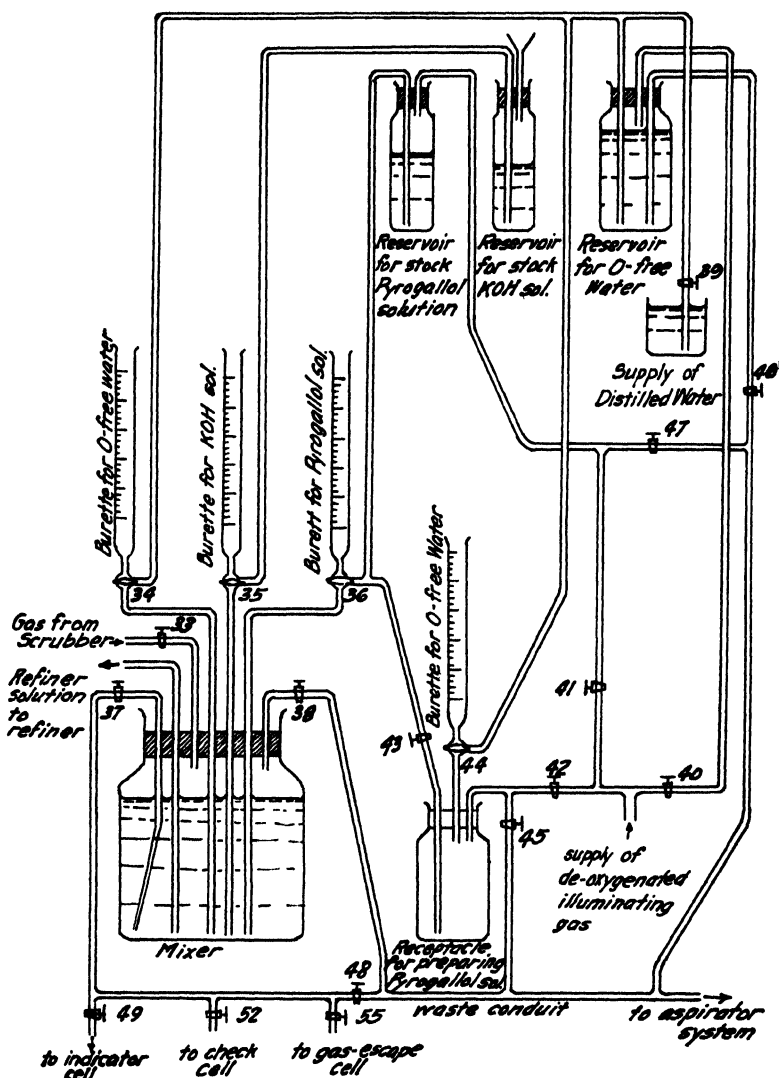


FIG. 9. Diagram showing arrangement of mixer and related parts.

work. De-oxygenated illuminating gas for this purpose is prepared by passing gas from the regular laboratory supply through several scrubber bottles arranged similarly to those used in connection with the nitrogen supply. In this case, however, because of lower pressure from the supply, the gas is passed through the chamber of each scrubber bottle without bubbling through the solution. A half hour of bubbling in the reservoir,

at the rate of 3 or 4 bubbles per second, the tube orifice being about 5 mm. in diameter, renders a liter of water sufficiently free from oxygen for subsequent use in the mixer. To start the siphons supplying de-oxygenated water to the two water burettes it is of course necessary to apply a little suction temporarily to each burette, letting the suction cease as soon as the siphon begins to operate.

Pyrogallol suitable for the indicator solution should not produce noticeable turbidity in the pyrogallol solution, nor should it produce in the completed indicator solution any color more intense than that of the palest standard-color solution to be employed. Powers-Weightman-Rosengarten Co.'s "Analytical" pyrogallol has been found to be satisfactory. Of course, commercial grades are used for the scrubber solution. Pyrogallol for indicator solution for the whole investigation should be of the same lot and the entire supply should be thoroughly mixed and then weighed into 50-gram charges, each hermetically sealed.

Stock pyrogallol solution is prepared in the receptacle therefor (a 500 cc. bottle), the stopper of which is fixed in position by its attachment to the three tubes that it bears. One of these tubes is connected with the reservoir for storing stock pyrogallol solution, another is connected with the burette for introducing measured quantities of de-oxygenated distilled water, and the third is connected with the aspirator system and also with the de-oxygenated illuminating gas supply. The reservoir for storing stock pyrogallol solution bears two tubes, one connected with (1) the de-oxygenated illuminating gas supply and (2) with the aspirator system, and the other with (1) a burette for measuring stock pyrogallol solution to be introduced into the mixer and (2) the receptacle in which the stock solution is prepared. Before stock pyrogallol solution is introduced into its storage reservoir, the air in the reservoir and connecting tubes is displaced by de-oxygenated illuminating gas. To accomplish this, cocks 41, 43 and 45 are opened, the air being drawn off through the aspirator system.

Preparation of stock pyrogallol solution proceeds as follows. The bottle receptacle therefor is removed from its stopper and set aside. Any water contained in the water burette leading into the receptacle is allowed to escape and the water that has stood in the tube leading from water reservoir to burette is allowed to be displaced by fresh water from the reservoir. Also, the air in the reservoir for stock pyrogallol solution is to be displaced by de-oxygenated illuminating gas, which is done by opening cock 41 for a time; gas from the reservoir is allowed to escape at the lower end of the tube that joins the lower receptacle with the reservoir above. Cock 43 is open during this process and is afterwards closed.

A charge (50 grams) of pyrogallol crystals is next introduced into the bottle receptacle for preparing pyrogallol solution and the receptacle is then

returned to its stopper. Air in the receptacle is next mostly removed through the aspirator system by temporarily opening cock 45. One-hundred cc. of de-oxygenated water is now added from the water burette, which has previously been freshly filled from the water reservoir (3-way cock no. 44). While the crystals are dissolving, the receptacle may be slightly agitated, which is possible on account of flexible rubber connections, and the process is hastened if a vessel of warm water is placed under the receptacle and then raised until the lower part of the latter is submerged. The partial vacuum over the pyrogallol solution in the receptacle is next satisfied by allowing the entrance of de-oxygenated illuminating gas through cock 42, which is then again closed. After this the newly prepared charge of stock pyrogallol solution is transferred to the reservoir for stock pyrogallol solution above. To do this, suction is applied in the reservoir by means of the aspirator system, through cock 47. Cock 43 is likewise open during this process, as is also cock 42, which allows the solution leaving the receptacle to be displaced by de-oxygenated illuminating gas. Cocks 42, 43 and 47 are now closed and cock 41 is opened to allow illuminating gas to satisfy the partial vacuum over the solution in the upper reservoir. The last mentioned cock is left open.

Aqueous pyrogallol gradually darkens somewhat on standing, but only slowly under the conditions provided in the reservoir for stock pyrogallol solution. The solution may stand in the reservoir for about two weeks, but when more than two weeks old it is generally best to discard what is left in the reservoir and replenish with fresh solution. To discard the contents of the reservoir, the receptacle bottle is removed from its stopper, cock no. 43 is opened, and the solution flows out by gravity through the same tube as that by which it was passed from the receptacle to the reservoir.

Potassium hydroxide solution is prepared in an open beaker by dissolving one pound of sticks in 500 cc. of distilled water. The purest product obtainable should be used for this purpose, with special precaution that it is free from iron. "Merck's reagent" has been found satisfactory. The solution is poured into the reservoir for stock potassium hydroxide, through a small funnel that penetrates the stopper. This reservoir stopper bears also a short tube of small bore, which serves as air vent, as well as the outlet tube that joins the reservoir with the hydroxide burette. No special arrangement is introduced for emptying the hydroxide reservoir, this being accomplished when necessary by removing the reservoir from its stopper. The solution may stand in the reservoir for a month or more without injury, since the openings to the atmosphere allow only very slow entrance of carbon dioxide and very little movement of water vapor. Experience indicates that this solution does not need to be guarded from atmospheric oxygen.

(d) PREPARATION OF THE INDICATOR SOLUTION.—To prepare an initial charge of indicator solution, air is first displaced from the mixer and its connecting tubes (see fig. 9). Some liquid is introduced into each of the 50 cc. burettes, which are connected with the mixer, from the corresponding reservoir. The siphons may be started by suction as mentioned above; the cocks 34, 35 and 36 are successively opened, and the tube leading into the mixer from each burette is filled, a small amount being allowed to enter the mixer itself.

Aspiration is next applied to the mixer through cock 38 and the mixer is then completely filled with water by successively filling and emptying the water burette, accomplished by manipulating the 3-way cock (34) at its base. Cock 38 is now closed and cock 37 is opened, so that the water just placed in the mixer is removed through the aspirator, refined nitrogen being simultaneously admitted through cock 33. This cock is then closed and the mixer is next rinsed by introducing and removing a small preliminary amount of the regular indicator solution. This is done by introducing into the mixer from the proper burettes, in the order given, 3 cc. of stock pyrogallol solution, 5 cc. of stock potassium hydroxide solution and 50 cc. of de-oxygenated water. While these solutions are being introduced, suction is continuously applied to the mixer through cock 38. This preliminary rinsing solution is then removed by the aspirator through cock 37, being replaced by refined nitrogen delivered through cock 33. One rinsing is found to be sufficient. Cock 33 is again closed and the regular charge of indicator solution is now prepared in the mixer just as was the rinsing solution, the amount used being 18 cc. of stock pyrogallol solution, 30 cc. of stock potassium hydroxide solution and 300 cc. of water. Finally the nitrogen cock 33 is opened. A freshly prepared charge of indicator solution should be allowed to stand in the mixer for a half hour or longer to insure complete mixing.

When the apparatus has been in operation and there is some old indicator solution in the mixer, that residue is of course first removed, just as was the water and afterwards the rinsing solution. While this is being done the burette cocks for the two solutions are manipulated so as to allow all solution that has been standing in the burettes or in the tubes leading to or from the latter to pass into the mixer. The mixer is next rinsed *at least three times* with small preliminary charges of indicator solution, in the manner described above, and the new charge of indicator solution is then prepared.

(e) THE COLOR STANDARDS.—As has been said, two different color-standard solutions are used in the colorimetric tests, one of a pale color corresponding to the color of the indicator solution at the beginning of the

test period and the other of a darker color corresponding to the color of the indicator solution at the end of the test period. In the present section these two standard-color solutions will be described, the pale one being called standard-color solution A and the darker one being called B.

The color-standard solutions are made by bringing together in acidulated water the proper proportions of the three colored salts, cobalt chloride, cupric chloride, and ferric chloride (2). The cobalt chloride used was of the Baker and Adamson Chemical Company's "Standard Purity, C. P." The other two salts were "Baker's Analyzed Chemicals," from the J. T. Baker Chemical Company. For dissolving them and for diluting the solutions acid water was used, this being prepared by adding hydrochloric acid solution having a specific gravity of 1.172 to distilled water in the proportions of 1 cc. of the acid solution to 99 cc. of water. A stock solution of each salt was first prepared by dissolving 50 gm. of the material from the original container and making up to 500 cc. of solution.

The exact composition of each of the two standard-color solutions is shown in table I. It is to be noticed that solution B contains no ferric chloride.

TABLE I
COMPOSITION OF STANDARD-COLOR SOLUTIONS

COMPONENTS	COLOR-STANDARD A	COLOR-STANDARD B
Acid water	50 cc.	50 cc.
Stock solution of cobalt chloride	24 cc.	100 cc.
Stock solution of cupric chloride	8 cc.	20 cc.
Stock solution of ferric chloride	2 cc.	

(f) COLOR OF THE INDICATOR SOLUTION.—In these studies pyrogallol described as containing no gallic acid was used for preparing the indicator solution, and, as already mentioned, the color standards A and B match respectively a light and a dark shade of this solution as it changes color with absorption of oxygen from the gas stream. The fresh indicator solution, as viewed in the indicator cell before any oxygen has been absorbed, is almost colorless. With the absorption of oxygen its color alters through light orange-browns (a stage of this region being matched by standard A), darker browns, reddish browns (a stage of this region being matched by standard B), wine red, and finally becomes nearly black. If the indicator solution fails to match either of the standards at the corresponding stage of its color change, this may be due to impure materials used in preparing the indicator solution itself or to substances acquired from the rubber tubing connections. In the last case the indicator solution may assume purplish tints as it ab-

sorbs oxygen. It is convenient to control the liquid inlet of the indicator cell with a Hoffman tubing-clamp on a rubber tubing connection, and the possibility of solution contamination from this source may be entirely obviated (1) by using tubing that has been dipped in hot paraffin, so that the solution does not come directly in contact with the rubber, and (2) by allowing solution to flow through the indicator cell, in recharging the latter, until none remains that has stood longer than 20 minutes in the connection. Of course the rubber connection might be replaced by glass tubing and a glass cock, but glass cocks are apt to be troublesome, when used for alkaline solutions.

5. ADDITIONAL NOTES ON THE METHOD AND APPARATUS

A few disconnected, additional notes on operation are here given, which might not be so readily understood if they had been inserted in the preceding sections.

CALIBRATION OF THE ABSORBERS AND INTERPRETATION OF INDEX VALUES.—

As has been said above, as long as only a single absorber is used it is possible to make comparisons between the oxygen-supplying powers of the soil at different depths, etc., without considering the area of the absorbing surface of the absorber, for that surface then remains the same for all tests. Even under these conditions it must be remembered, however, that no test can be made for any absorber environment unless the absorber is capable of taking in oxygen through its porous wall just as rapidly as that substance is supplied by the environments to the outer surface. When exposed in environments of very high oxygen-supplying power (as the ordinary atmosphere, for example), any absorber always gives the same test time, which is really a measure of the ability of the absorber wall to transmit to the gas stream, oxygen that arrives at its external surface. The water-impregnated, porous-porcelain absorbers used in these studies all give test periods of less than 4 minutes when exposed to the ordinary atmosphere. There is no doubt that an absorber so exposed receives oxygen on its outer surface much more rapidly than is indicated by this short test time and it is clear that the length of this test time is not at all a measure of the oxygen-supplying power of ordinary air. It follows from this that, in seeking to determine the oxygen-supplying powers with different absorber exposures in the soil, etc., no account is to be taken of determinations with this type of absorber unless the test times are more than 4 minutes. When the test time is greater than 4 minutes it is indicated that the environment is not supplying oxygen to the outside of the absorber as rapidly as the absorber might deliver it to the gas stream if the rate of supply were greater. The environmental supplying power is, in such

cases, the limiting condition that determines the length of the test time, for we are sure that the absorbing power of the absorber is not taxed under these conditions. As long as this threshold rate of operation for the absorber itself is not reached or too closely approached, the various degrees of oxygen-supplying power of the several environments are to be considered as inversely proportional to the lengths of the corresponding test times.

Of course it is desirable that determinations made with different absorbers should be comparable, and this necessitates calibration of all absorbers with reference to their absorbing surfaces. In the work here reported, as many as eight absorbers have been employed at once, with the gas flowing continuously through all of them, although determinations for some of them were made only at long intervals. The employment of several absorbers, whether simultaneously or not, immediately introduces the requirement for absorber-standardization or calibration; for no two absorbers may be expected to have exactly the same ability to deliver oxygen to the gas stream under any given set of environmental conditions. Absorbers may differ with respect to this ability in two ways. (1) The amount of surface exposed to the environment may be larger for some than for others. (2) Absorbers may differ with respect to average thickness of wall, etc., that is, with respect to the average permeability to diffusing dissolved oxygen. As has been said, all absorber cones used in these studies were so prepared that they gave, when exposed to the ordinary atmosphere at room temperatures, test times of less than 4 minutes and the external surfaces exposed were nearly alike. Since the oxygen-supplying powers of the environments to be tested were practically always such as to give test times of more than the 4-minute limit, no attempt has yet been made to secure calibration coefficients with respect to the average permeability of the absorber walls. The area of the absorbing surface exposed by each absorber was carefully determined, however, by measurement and calculation. The results of these calibrations were always used when determinations secured by means of different absorbers were to be compared.

During the progress of these studies absorbers have remained undisturbed in various soil environments, in the laboratory and out-of-doors, for periods of a year or more and none have given any evidence of sufficient alteration of the absorber wall to impair the ability of the latter to operate properly. All absorbers tested in air after long exposure to the soil have supplied oxygen to the gas stream at a sufficiently rapid rate to give the standardization test time of four minutes or less.

To be acceptable in this work an absorber must have given in ordinary atmosphere a test time less than 4 minutes, as has been said. When the cones are in operation in environments that are to be compared with respect

to their oxygen-supplying powers, the test time for each determination is first to be compared with its calibration test time in air. If the former is not greater than the latter the determination in question is regarded as beyond the capacity of the apparatus; all that can be said is that the oxygen-supplying power of the environment thus tested is about equal to or greater than the absorbing capacity of the instrument. In soil tests this result can be secured only with extremely well aerated soils and with very shallow depths, and the oxygen-supplying power of the soil in such cases may be safely considered as ample for the health of any plant. It is soil environments of low oxygen-supplying powers that are mainly interesting when determinations of these powers are being compared.

Test times for different absorbers in actual determinations may not be directly compared, even when each of them is greater than the corresponding calibration test time, since the external areas of the absorbing walls are generally not exactly alike. Nor can the reciprocals of the determination test times be directly compared if secured with different absorbers. These reciprocals may be termed indices of oxygen-supplying power. It is necessary, before comparing them, to correct each one by dividing by the actual area of the absorbing cone with which it is secured. The corrected indices, which are directly comparable, are denoted by the formula $\frac{1}{ta}$, where t is the test time in minutes and a is the area in sq. cm. of the corresponding absorber.

To illustrate these points, an absorber with an external porous surface of 64.0 sq. cm. (a) gives in a certain soil environment a test time of 70 minutes (t) and the corrected index of the oxygen-supplying power of the given environment is consequently $\frac{1}{64 \times 70}$, or $\frac{1}{4480}$; while another absorber with an area of 65.64 sq. cm. (a') gives a test time of 160 minutes (t') and the corrected index for the second environment is consequently $\frac{1}{65.64 \times 160}$, or $\frac{1}{10502.4}$. The oxygen-supplying power of the first environment is shown to be 134 per cent. greater than that of the second. Both t and t' must be greater than 4 minutes with the absorbers used in the work here reported.

For simply comparative purposes it is not necessary to express the indices in terms of ordinary units of oxygen; any environment may be compared with any other after the manner of the illustration just given, and the oxygen-supplying powers of any number of environments may each be expressed in terms of that for some selected environment, the latter considered as unity. In physiological investigations it may, however, be desirable to express the index of oxygen-supplying power as a number of milli-

grams of oxygen that might be delivered per hour through, or across, a square meter of absorbing surface. To accomplish this it is necessary to determine the exact value, in milligrams, of the oxygen unit (u) of the colorimetric determination. By several tests, the oxygen unit of the present studies was found to be approximately 0.03 mg. That is, the solution in the indicator cell absorbed 0.03 mg. of oxygen while changing in color from that of color standard A to that of standard B. The value of u would of course be different if the indicator solution were different from the one here used or if it were employed in different amount; or if either of the color-standard solutions were different from the corresponding one described above.

Turning again to the general formula for the relative index value, it is seen that, for any determination, u mg. of oxygen is supplied in t minutes through a surface of a sq. cm., a rate of supply equivalent to $\frac{u}{ta}$ mg. per minute per square centimeter of absorbing surface. Through the surface of a square meter the corresponding maximum supplying power of the given environment would be $\frac{10,000 u}{ta}$ mg. per minute, and that of a square meter of surface would be $\frac{600,000 u}{ta}$ mg. per hour. In the illustration given above, the first environment can deliver $600,000 \times 0.03 \times \frac{1}{4480}$, or 4.02 mg. of oxygen per square meter per hour $\left(\frac{600,000 u}{ta} \right)$ and the second can deliver $600,000 \times 0.03 \times \frac{1}{10502.4}$, or 1.71 mg. $\left(\frac{600,000 u}{t'a'} \right)$. To express any index value in terms of milligrams per hour per square meter of surface, we simply multiply the relative index value $\left(\frac{1}{ta} \right)$ by $600,000 u$; or, since the value u is 0.03 mg., by 18,000; we thus change the expression from colorimetric units of oxygen per square centimeter per minute, to milligrams of oxygen per square meter per hour. In reporting the experimental results below, the index values will be given in terms of milligrams of oxygen per square meter per hour.

TESTING THE APPARATUS BEFORE INTRODUCING SOLUTION.—The complexity of the apparatus makes it desirable to test its operation after all parts have been assembled, using distilled water instead of solution. After it has been observed that the pressure and orifice controls are functioning properly, and no difficulty is experienced in any of the necessary manipulations, the various solutions may be introduced and actual operation may be undertaken.

ADJUSTMENT OF THE THROTTLE-CONTROL.—Occasionally it becomes necessary to draw off a liter or more of nitrogen from the scrubber while the apparatus is in operation, as in recharging the refiner tubes with fresh solution or in discarding aged indicator solution from the mixer. This may be done in the following manner without seriously altering the established gas pressures in the scrubbers. The rate of bubbling of nitrogen as it enters the first scrubber unit is determined, then the throttle-control valve is opened sufficiently to allow gas to escape freely from the secondary pressure-regulator. Nitrogen is then passed into the part of the apparatus where needed, care being taken that the movement is not rapid enough to reduce the gas pressure in the scrubber below the point where gas ceases to escape from the pressure control. After this has been accomplished the normal rate of flow of gas from the pressure cylinder is re-established by again stopping down the throttle-control valve until the former rate of bubbling in the first scrubber unit is resumed.

USE OF THE ASPIRATOR SYSTEM.—For many purposes gravity and the gas pressure in the different containers are sufficient to move the solutions from one part of the apparatus to another as desired, and it is generally better to employ these forces when they suffice than to use suction through the aspirator, which is apt to be uneven and sometimes violent. For example, it is best not to use the aspirator for removing and introducing solution in operating the cells of the indicator system. As has been indicated, the aspirator is necessary in many cases, however, but when it is used care should be exercised not to allow pressure gradients to become unnecessarily great. Excessive pressure differences may strain the apparatus at many points; air may be caused to enter through glass cocks, that are otherwise tightly closed, mercury may be drawn from the mercury seals into the tubes, etc. If aspirator suction is ever applied to the indicator cell, care must be taken that mercury is not drawn from the selector up into the lead tube leading to the indicator cell; a brief contact of mercury with the tube wall would ruin the tube by amalgamation. Other similar precautions might be mentioned, but the operator who has assembled the apparatus will be familiar with its weaker and stronger points and will be guided by that familiarity.

RUBBER-TUBING CONNECTIONS.—While glass or metal cocks might well be used throughout the apparatus, ordinary rubber connections with Hoffman tubing clamps have been found to serve well except where glass cocks have been specified. The pieces of rubber tubing should first be dipped in hot melted paraffin, which greatly increases the imperviousness of their walls to oxygen diffusion. For the burettes connected with the mixer system it is advisable to use clamp-controlled rubber connections between each burette

and its supply reservoir and between the burette and the mixer. Each burette is furnished with the usual 3-way glass cock, however, and when a burette is to be used the proper clamps are first opened, allowing the flow of liquid to be controlled by the three-way cock. When the burette is not in use the clamps are kept closed, to prevent any possibility of leakage past the cock. Furthermore, it is occasionally necessary to remove the plug of the glass cock for cleaning or greasing, and the closed clamps make this possible without risk of air being admitted to the tubes or of solution being wasted.

6. GENERAL APPLICABILITY OF THE NEW METHOD

While the method described in the preceding pages has been worked out for the purpose of comparing soil environments as they may influence plant growth, it should be suitable for studies of any environments that supply oxygen to an oxygen-absorbing surface at slow rates. Modification of the apparatus here described may doubtless make it available for studies of environments that offer more rapid rates of oxygen supply. Also, the apparatus and general method here employed should, with suitable changes, be useful for studying supplying powers with reference to other gases, such as carbon dioxide.

IV. Experimentation

1. EXPERIMENTS WITH SOILS

Some experimental tests of the oxygen-supplying powers of soils have been carried out, partly with the apparatus described in the preceding section and partly with the preliminary form described by HUTCHINS and LIVINGSTON. The results of these tests are presented below as experiments numbered from 1 to 6, inclusive. The first three experiments were carried out with the original apparatus, the standardization of which was not as nearly perfect as is that of the improved apparatus described in the present paper. The remaining experiments were performed with the new apparatus. Of the first three, the data of number 1 were used in the preliminary publication on this subject by HUTCHINS and LIVINGSTON. Experiments 2 and 3 were performed with the earlier apparatus but are now recorded for the first time. Since no tests were made to compare the oxygen-absorbing efficiency of the two forms of apparatus, it is best in the case of these three experiments to neglect the actual numerical values, as published for the present experiment 1 in the HUTCHINS and LIVINGSTON paper, regarding them merely as indications of relative magnitudes and without attempting detailed quantitative comparisons between them and the

corresponding values secured by means of the improved apparatus. For experiments 4, 5 and 6 of the present series, the quantitative values based on the more nearly perfect standardization of the improved apparatus will be considered.

EXPERIMENT 1.—The first soil experiment, as mentioned above, has been somewhat fully described in the preliminary paper on this subject. An outline of the essential features and results of this experiment follows. A single, previously tested absorber was placed in a box of loosely sifted, moist soil, 8 cm. from the bottom and 7.5 cm. from the top. During the first two days of the experiment, the oxygen-supplying power indicated by the absorber gradually decreased and was then maintained with but negligible fluctuation for over forty days. An additional 8 cm. layer of loosely sifted, moist soil was then added to the box, thus bringing the absorber to a depth of 16 cm. below the soil surface. A lengthening of the test time was evident after three hours and continued during the next three days, after which the oxygen-supplying power was shown to be about 76 per cent. of the original maintained value just mentioned. Water was next added to the soil, corresponding to 6 cm. of rainfall and nearly saturating the soil. The resulting soil shrinkage lowered the surface so that the absorber was now only 13.5 cm. below the surface. Eight hours later the supplying power was found to be about 46 per cent. of the original value and the same continued to hold for 18 hours. Then the soil was firmly packed, thus bringing the absorber to within 11 cm. of the soil surface. Immediately after packing had been completed, an oxygen-supplying power 32 per cent. as great as the original was indicated, and another determination beginning four hours after the packing and lasting 84 hours gave a supplying power only 2 per cent. as great as the original.

If the index of oxygen-supplying power for a depth of 8 cm. in loose, moist soil is taken as 100, then the corresponding power for a depth of 13.5 cm. of firmly packed, nearly saturated soil, was only 76. Adding 8 cm. of loosely sifted soil diminished the relative value of the oxygen-supplying power at the absorber surface to 46. The latter value was still further decreased to 32 when the soil was thoroughly wetted, and to 2 when it was wetted and firmly packed.

EXPERIMENT 2.—In the second soil experiment an absorber was centrally placed in moist soil held in an ordinary porous flower pot of the 9-inch size, with the roots of a healthy *Pelargonium* plant lying in the immediate neighborhood of the absorber surface. The oxygen-supplying power of the soil mass was studied in relation to alternate wetting and drying of the soil. The absorber used was of the conical type, but somewhat larger and with thicker walls than are specified in the description of the apparatus

given in earlier parts of this paper. Twenty-four hours after the beginning of the experiment the index of oxygen-supplying power was determined. The pot was then thoroughly watered from above in the ordinary way and was placed with its base in water to a depth of 2 cm. After 17 hours in this position the index value was only 42 per cent. as great as in the original determination. The pot was then removed from the water, and drainage and evaporation were allowed to proceed freely for 30 hours, during which period tests were frequently made, the index value gradually increasing to 89 per cent. of the original. The soil mass was next thoroughly saturated by temporarily plunging the pot into water, and the pot was then allowed to drain with its base standing in a 2 cm. water layer. When drainage had ceased the index value was found to be 53 per cent. of the original. The preparation was then removed from the water and freely exposed to the air for 18 hours, at the end of which period the index had risen to 80 per cent. of the original. The soil was next packed enough to lower its surface in the pot about 2 cm., which of course increased the soil water content with reference to the soil volume. A test 15 hours later gave an index value of 47 per cent. of the original. For the next 50 hours evaporation was allowed to proceed, the water content of the soil gradually decreasing and the index value gradually increasing, until the latter became once more 80 per cent. as great as the original value.

EXPERIMENT 3.—The third soil experiment was similar to the second, with a 9-inch flower pot of garden soil and a centrally placed absorber, but without any plant. Beginning with moist, loosely packed soil, the index of oxygen-supplying power was determined five hours after starting the experiment. The pot was next stood with its base in water to a depth of 2 cm. for 44 hours, during which period the index of the oxygen-supplying power of the soil at the surface of the absorber decreased gradually, as shown by frequent tests, to 15 per cent. of the original determination. The pot was then removed from the water and a strong air current from an electric fan was directed against the pot wall for 40 hours, to hasten evaporation. During this 40-hour period the index of the oxygen-supplying power of the soil steadily increased and at the end of the period it was 88 per cent. as great as the original. The fan was then stopped and the pot was thoroughly watered in the usual way, being again placed with its base in water. Frequent subsequent tests indicated a rapid decrease in the oxygen-supplying power, and a final test after 24 hours gave an index value of 13 per cent. as great as the original value.

The second and third soil experiments show in a general way what may be expected to occur in the case of an ordinary potted plant in a greenhouse or living room, when thoroughly watered once or twice a day. When the

soil of such a pot is only ordinarily moist the oxygen-supplying power of the root environment may be from 2 to 6 times as great as it is immediately after a thorough watering, in spite of the fact that the water added contains much dissolved oxygen.* The index value fluctuates greatly with alterations in the soil moisture content, and it is greater when the soil is loose and less when the soil is more thoroughly packed. It seems probable that ordinary pot-grown plants would be more vigorous if the oxygen-supplying power of the soil might be maintained as high as possible, without allowing the water supply to the roots to become inadequate. To accomplish this it might be desirable to add water to the pot in small amounts and at frequent intervals, but the soil condition here thought of can probably be well maintained in such cases by some automatic water-supplying device such as the LIVINGSTON (11) porous-porcelain auto-irrigator.

EXPERIMENT 4.—The fourth soil experiment, and the fifth and sixth also, were carried out with the improved apparatus as has been mentioned, and also with a much larger soil mass than was used in the three cases already considered. A moist, sifted garden soil, similar to that used in the previous experiments, was used. It was held, with medium packing, in an upright, galvanized, sheet-iron cylinder 60 cm. high and 40 cm. in diameter, tight excepting that the top could be opened and that there were three small holes through the wall close to the bottom, these also being opened or closed according to need. Several absorbers were placed in the vertical axis of the cylinder at different depths. The two lead tubes for each absorber were led spirally outward through the soil to the wall of the cylinder and then upward, this precaution being taken to avoid gas interchange with the air above, along the outer surface of the tubes.

With alterations in the packing and moisture content of the soil mass it was found, as is generally true with such cylinders of soil, that a considerable annular air space soon developed just within the cylinder wall, especially near the top. Such separation of the soil from the container wall must of course allow very free air movement in its region, which might bring atmospheric oxygen to the lower absorbers without its having traversed the soil above. To care for this opening was found to be one of the most important problems of technique in such experiments as these. After

* HOWARD and his co-workers (10), especially p. 196, have emphasized what may be an important point with reference to precipitation and irrigation, as these are related to soil aeration, namely that rain is generally more beneficial to plants than is flood irrigation of the same amount. The idea is advanced that this may be due to the fact that natural precipitation supplies the soil with water that is highly charged with oxygen, this water percolating to great depths and tending to carry much of its dissolved oxygen with it. These authors have given us much valuable information and discussion on the importance of soil oxygen in agriculture and forestry.

preliminary attempts of various kinds, it was found that this difficulty could be entirely obviated by the introduction of a seal along the surface of contact between soil and cylinder, in the upper region. The best material tried for making this seal was a three-per cent. agar gel containing a small amount of copper sulphate as an antiseptic. In these experiments the cylinder of soil was allowed to stand for several days before the seal was applied, and then the hot liquid agar was simply poured around the edge of the soil surface. Agar was added until downward flow along the wall of the cylinder ceased. The agar solidified within 10 or 12 hours and there resulted a very excellent seal which was found to prevent atmospheric oxygen from finding its way downward at the surface of contact between soil and cylinder. This use of agar gel is in some respects similar to the use of paraffin in the WHITNEY paraffined wire pot for biological tests of soil productiveness (12, 20). Agar was found to be more satisfactory than paraffin in the present work.

In the fourth soil experiment four absorbers of the new conical type described in the early part of this paper were used, being placed 10, 20, 30 and 45 cm. below the soil surface. Since, as has been said, the improved apparatus was used in this and the two following experiments, it will be possible to employ the actual values of the supplying power index in reporting the results in all three cases.

The bottom openings of the cylinder were closed in this experiment. After the test time had been determined for each absorber (the index values being, respectively, 25.3, 14.2, 4.3, and 1.7 milligrams per square meter per hour), the top of the cylinder was closed by means of a disk of tinned sheet-iron resting on the soil, with a paraffined seal around the margin. During the next 40 hours frequent determinations were made for each of the four absorbers. The index value of the oxygen-supplying power gradually decreased in every case, until all four index values were below 0.4. Then the top of the cylinder was reopened and determinations were continued during the next three days. The index value for each absorber gradually increased until it attained its initial magnitude.

These results show clearly that the oxygen-supplying power of such a soil mass as that used in this experiment, open at the top but otherwise cut off from the atmosphere, is, as might be expected, relatively high near the soil-air surface and progressively lower as we proceed downward. In this particular experiment the index value for a depth of 45 cm. was found to be only 6.7 per cent. of the corresponding value for a depth of 10 cm. Since all of the oxygen reaching any absorber must have passed through the soil above, the rate of arrival of oxygen at the level of one of the deeper cones must be lower than in the case of a cone nearer the soil sur-

face. To reach the uppermost cone, atmospheric oxygen had to diffuse through only 10 cm. of soil, while it reached the lowest cone only after traversing 45 cm. of soil. The rate of arrival of oxygen at any absorber might therefore be expected to be roughly related to the depth of the cone. As a porous, water-impregnated body, the soil offers resistance to the diffusion of oxygen but it must be remembered that the soil also acts as a reducing agent and that the soil materials and soil organisms are continuously absorbing oxygen, which feature must in effect increase the resistance offered to downward movement of oxygen.

It is interesting to note that closing the top of the cylinder in this experiment rapidly brought all four indices to values much lower than that indicated for the deepest absorber when the top was open. Entrance of oxygen from the air ceased with the application of the upper seal and it is evident that soil processes, such as oxidation and the respiration of organisms, then rapidly used up most of the oxygen that was in the soil at the time of closing.

EXPERIMENT 5.—The fifth soil experiment was performed with a cylinder of soil like that used in the fourth experiment. After the soil had been placed in the cylinder it was thoroughly saturated with water, which was allowed to enter through tubes attached to the three lateral openings near the bottom of the cylinder, the water flowing in slowly and displacing the soil gases until the free water surface appeared above the soil surface. The soil mass was then allowed to drain by opening the three outlets at the bottom. The marginal agar seal was introduced after drainage had ceased, and then the soil surface was covered with a layer of sand to a depth of three centimeters, the sand surface remaining exposed to the air throughout the experiment. Only three absorbers were used in this instance, these being 14, 32, and 42 cm., respectively, below the bottom of the sand layer. Determinations of the oxygen-supplying power were made after the agar seal and sand mulch had been introduced. The index values for the three absorbers, in the order given, were found to be 11.3, 2.3 and 0.08, respectively, and these values were maintained for a period of nine days. An experiment on the germination of wheat seed at different depths was carried out simultaneously with the determination of these index values. The results of this germination experiment will be considered in the next section.

In the case of this wet soil, it is seen that the index values are again lower for greater depths and that the observed differences are more pronounced than in the case of the moist soil of the fourth soil experiment. With the possible exception of the upper few centimeters of the soil mass, it appears that the oxygen-supplying power of the very wet soil for any given depth was much lower than for the same depth in the moist soil of the fourth experiment.

EXPERIMENT 6.—The sixth soil experiment was like the fourth, excepting that the surface of the moist soil was covered with a sand mulch similar to that used in the fifth experiment and the cover used for closing the top of the soil cylinder in the present instance was perforated with a number of holes, 5 mm. in diameter, which were initially closed with wax. No determinations of oxygen-supplying power were made until after the cylinder had been completely closed. As soon as the preparation was complete, however, determinations for the four absorbers were begun and these were continued at intervals for a period of two and one-half days. Then seventeen of the openings in the cover, equally distributed, were opened, admitting air at a limited rate, and determinations were continued seven days longer. Germination tests with a number of different kinds of seeds were carried out in this cylinder of soil, and the results of these will be described in the next section. The index values for critical stages of this experiment are shown in table II.

TABLE II

CHANGES IN INDEX OF OXYGEN-SUPPLYING POWER IN SOIL CYLINDERS WHEN CLOSED AND ON OPENING THEM

DEPTH OF ABSORBER IN CENTI- METERS	INDEX OF OXYGEN-SUPPLYING POWER (MG. PER SQ. M. PER HOUR)				
	Immediately after closing cylinder	1 to 2 days after closing cylinder	2 to 2.5 days after closing cylinder	4 days after opening cylinder	7 days after opening cylinder
10	>75.0	0.9	<0.2	15.4	22.6
20	>75.0	0.9	6.2	7.4
30	>75.0	<0.2	...	5.6	2.3
45	>75.0	<0.2	<0.2	2.0	0.5

Immediately after sealing, all absorbers indicated index values of at least 75.0. When the cylinder had been closed for from one to two days all index values had decreased to 0.9 or lower, and during the following half-day the upper and lower absorbers both showed clear indications that their index values were less than 0.2. In the two and one-half day period during which no oxygen was admitted from without, the soil processes and germinating seeds had apparently removed almost all of the oxygen initially enclosed, and the oxygen-supplying power of the soil was consequently very low for all depths.

Removing the seals from the openings in the cover was of course followed by rapid entrance of oxygen from the atmosphere, and after the cylinder had been open for four days the index values ranged between 15.4 and 2.0, as shown in the table. In the following three days the index for the upper absorber increased from 15.4 to 22.6 and that for the second also increased slightly (from 6.2 to 7.4). On the other hand, the indices for the lowest two absorbers decreased during the same three days, from 5.6 to 2.3 for the third absorber and from 2.0 to 0.5 for the fourth. The reversal noted for the two lower absorbers is probably due to germination of the seeds in the soil above them and to the consequent rapid increase in the absorption of oxygen by seedling respiration in the upper soil layers.

SUMMARY OF SOIL EXPERIMENTS.—For the soils used in these tests, when only the upper surface of the soil mass was exposed to the atmosphere, as is generally true for soils in the field, the oxygen-supplying power is shown to be greater near the air-soil surface and smaller for greater depths. It is also shown to be greater, for the same depth, in dryer or less firmly packed soil and smaller for wetter and more firmly packed soil. Of course the actual values of the supplying-power indices should be different not only with regard to depth, moisture content and firmness of packing, but also with respect to the kind of soil dealt with (whether sandy or loamy, etc.) and with respect to the prevalence of aerobic organisms and of readily oxidizable materials in the soil between the absorber and the air-soil surface. Soil temperature, barometric pressure and other climatic conditions may be expected to be more or less influential.

2. EXPERIMENTS WITH SEED GERMINATION

OXYGEN-SUPPLYING POWER REQUISITE FOR SEED GERMINATION.—The germination of seeds furnishes an excellent example of very rapid and vigorous growth, with correspondingly high respiration rates. It is known that the seeds of some plants are able to germinate with very low rates of elementary oxygen supply, while others fail to germinate at all excepting with an abundant supply of elementary oxygen. On account of the ease with which many kinds of seeds may be tested for germination in different environments, some time was devoted in these studies to some preliminary determinations of the approximate oxygen-supplying powers necessary for germination in a few different kinds of seeds. The few experiments that have been performed along this line will be briefly noted below, simply as illustrations and suggestions. The method and apparatus used for determining oxygen-supplying power in all these experiments were of the improved form described in the earlier part of this paper, and the supplying-power indices dealt with below are all expressed in terms of milligrams per hour per square meter of absorbing surface. These values are comparable with those given above for soil experiments 4, 5 and 6.

GERMINATION EXPERIMENT 1.—In the first germination experiment, seed of *Triticum aestivum* (wheat) was first thoroughly soaked in water for 24 hours at room temperature, and was then packed into a sheet metal cylinder similar to the one used for the experiments with soils described above. As the seed was placed in the cylinder, absorber cones were centrally placed, one above the other, after the manner followed in the soil experiments. Twenty-four hours after the placing of seeds and absorbers, germination had just visibly begun to a depth of about 30 cm. and absorbers at depths of 15 cm. and 30 cm. showed index values of 14.1 and 2.4, respectively. At the end of an additional 24 hours germination was well advanced to a depth of about 15 cm. (with rootlets a centimeter or more long), while seeds at greater depths showed no further germination than they had shown at the end of the first 24 hours. After the second 24 hours the upper cone, 15 cm. deep, gave an index value of 1.5 and the second cone, 30 cm. deep, gave one of less than 0.05. Of course all seeds were well aerated when first placed in the cylinder, but those in the upper region must have absorbed oxygen at a very rapid rate, thus taking up most of the oxygen reaching them, and preventing the deeper seeds from getting oxygen at any considerable rate from the air above. Excepting for the first hour or so after the beginning of the experiment, the seeds nearest the surface of the seed mass naturally received the most rapid supply of oxygen, as is shown by the determinations after the first 24 hours. The avidity with which the actively germinating seeds absorbed oxygen apparently increased very rapidly after that time. During the second 24-hour period all seeds germinated well and rapidly down to a depth that showed, at the end of this second period, a supplying power only about one-seventh of the corresponding supplying power at the end of the first 24 hours. It is safe to say that good germination occurred with supplying powers of more than 1.5 mg. per square meter per hour (starting with 14.1 mg. and ending with 1.5 mg.) during the period. The average index value for the second 24 hours, during which active germination occurred, was about 3.0 for the lower depth at which germination was active, and this average value may be regarded as about the lower limit of oxygen-supplying power that would permit good germination. During the first 24 hours, germination began in the region of the seed mass that had oxygen-supplying powers of less than 2.4 mg. per square meter per hour. This indicates that the lower limit of oxygen-supplying power for the germination of a well-watered wheat seed lies between 3.0 mg. and 2.4 mg.; this lower limit seems surely not to be lower than 1.5. Seeds at greater depths than 30 cm. failed to make any visible beginning of germination, and the index values for these deeper regions of the seed mass were all less than 0.05. Of course these conclusions are based only on this particular experi-

ment; with non-oxygen conditions different from those that obtained in this particular cylinder of seed it may be supposed that the environmental oxygen-supplying power requisite for the rate of germination here considered would have had a value more or less different from the mean index value here given.

GERMINATION EXPERIMENT 2.—As has been mentioned, some seed germination tests were made in connection with soil experiment 5 described above. The results of these tests will now be considered as experiment 2 on seed germination. Seeds of the following plants were used: *Triticum aestivum* (wheat), *Zea* (field maize and Navajo maize), *Oryza sativa* (rice) and *Gossypium hirsutum* (cotton). The unsoaked seeds were planted at depths of 2, 14, 24, 34, and 44 cm., the second, fourth and fifth depths being in the vicinity of the oxygen absorbers used. To facilitate the recovery of the seeds from the soil, one piece of paraffined bobbinet was placed 2 cm. below, and another at an equal distance above, each seed layer. As mentioned in the description of soil experiment 5, the completed preparation was saturated with water, drained and allowed to stand nine days. At the end of that period the seeds were examined. At the 2-cm. depth, seeds of all five kinds had germinated and produced vigorous shoots. At a depth of 14 cm., and with an index of oxygen-supplying power of 11.3, field maize, wheat and rice had produced vigorous shoots, while Navajo maize had produced only small roots and cotton had not visibly germinated. At the 24 cm. depth the oxygen-supplying power was not measured, but it was surely between 11.3 mg. (that for the 14-cm. depth) and 2.3 mg. (that for the 34-cm. depth), probably about 4.5 mg. per square meter per hour. Field maize, wheat and rice at this depth produced small roots only, while Navajo maize and cotton failed to show any germination. At the 34-cm. depth, with an index of oxygen-supplying power of 2.3, as well as at the 44-cm. depth, with an index of 0.08, rice alone germinated.

From these results it appears that the environmental oxygen-supplying power necessary for healthy germination of wheat is greater than 2.3 mg. per square meter per hour, and lower than 4.5 mg. This conclusion agrees very well with that derived from the results of experiment 1, in which this limit was estimated as about 3.0 mg. per square meter per hour. The results of experiment 2 indicate also that the minimum oxygen-supplying power necessary for healthy germination is different for different kinds of seeds. The minimum requirement for field maize seems to be similar to that for wheat, while the minimum requirement for rice is shown to be very much lower, and those for Navajo maize and cotton appear to be considerably higher.

GERMINATION EXPERIMENT 3.—The third germination experiment was carried out in connection with soil experiment 6, described above, and was

so planned that the germination of several kinds of seeds at different depths in the soil mass could be studied in connection with the indices of environmental oxygen-supplying power for those depths. Sixteen kinds of seeds were used, being so placed in the soil that a number of seeds of each kind were at each of the following depths: 2, 10, 20, 30, and 45 cm. The last four depths correspond to the depths of the four oxygen absorbers.

To facilitate final observation, the seeds were planted in soil rather firmly packed in special rectangular chambers measuring 8 cm. long, 2 cm. wide and 1.5 cm. deep, open at the top and closed at the bottom with paraffined bobbinet, the lateral walls being of paraffined cardboard. Each chamber served for four (smaller) or two (larger) kinds of seed.

The cylinder was sealed immediately after the placing of the soil, seed-chambers and absorbers. The indices of oxygen-supplying power for the four absorbers all decreased during the next 60 hours to values below 0.2. Then the perforations in the cover of the cylinder were opened so as to admit oxygen at a limited rate, and the values of the indices for all absorbers increased until the index ranges for the three-day period from the fourth to the seventh day were 15.4–22.6 for the 10-cm. depth, 6.2–7.4 for the 20-cm. depth, 6.6–2.3 for the 30-cm. depth and about 0.5 for the 45-cm. depth. The seeds were not soaked before planting and it may be supposed that they had not taken up sufficient water for germination until after the supplying-power rates indicated by these index values had been established in the soil around them.

Seeds of *Gossypium hirsutum* (cotton), *Brassica oleracea* var. *capitata* (cabbage), *B. nigra* (mustard), *Trifolium repens* (white clover), *T. incarnatum* (crimson clover), *Phleum pratense* (timothy) and *Melilotus alba* (sweet clover) germinated well in damp-chamber tests but failed to show visible germination at any of the depths tested in the soil. Apparently these seeds all require, under the other conditions of this experiment, an oxygen-supplying power of not less than 22.6 mg. per square meter per hour. *Hibiscus esculentus* (okra) seed germinated at depths of 2, 10, and 20 cm. but failed to show germination at depths of 30 and 45 cm., the supplying-power requirement for germination being in these cases apparently greater than 5.6 mg. per square meter per hour. Seeds of *Cucumis melo* (muskmelon), *Medicago sativa* (alfalfa) and *Lycopersicum esculentum* var. *validum* (tomato) germinated well at depths of 2, 10, 20, and 30 cm., the minimum oxygen-supplying power necessary for germination of these seeds being apparently not greater than 2.0 mg. per square meter per hour. Seeds of *Trifolium hybridum* (alsike clover), *Oryza sativa* (rice) and *Zea* (field maize, Navajo maize and popcorn) germinated at all depths tested, the minimum oxygen-supplying power necessary for germi-

nation being apparently considerably less than 2.0 mg. per square meter per hour in these cases.

In general the enlargement of the seedlings, where visible germination occurred, took place less rapidly for seeds at the greater depths and more rapidly for those nearer the soil surface. At the time of observation, for example, wheat shoots from seeds at a depth of 2 cm. were about 21 cm. long, while those from seeds 20 cm. deep were about 10 cm. long and the seeds 45 cm. deep exhibited no shoots but bore roots about 2 cm. long. These observations suggest that the early growth of seedlings may exhibit relations to environmental oxygen-supplying power somewhat similar to, but different in detail from, the corresponding relations indicated for germination, and that shoot growth and root growth may in some cases, at least, manifest different relations to oxygen-supplying power.

GENERAL DISCUSSION OF THE GERMINATION EXPERIMENTS.—It is interesting to note that the results of the third germination experiment are consistent with those of the other experiments on germination as far as indications regarding the oxygen-supplying power requirement for the germination of wheat are concerned. Taking the results of the three experiments together, it may be said that the oxygen-supplying power necessary for the germination of wheat seed when well supplied with water and at room temperature lies within or about the range between 3.0 mg. and 2.3 mg. per square meter per hour. It appears from the results of germination experiments 2 and 3 that field maize has a minimum similar to that of wheat, although observations of the seedlings suggest that the minimum for growth of the former may be somewhat lower. Cotton seed appears to require a much higher rate of oxygen-supplying power (perhaps greater than 22.6 mg. per square meter per hour), while the minimum requirement for the germination of rice seed is clearly very much lower than that for the germination of wheat, surely lower than 0.5 mg. per square meter per hour.

As has been said in the introduction to this paper, very little study has as yet been devoted to the quantitative aspect of the oxygen requirements of plant roots. LIVINGSTON and FREE and also CANNON have published a few notes on the general relations between the health and growth of plants and the oxygen content of the soil about their roots. Their published results are summarized here for the sake of comparison.

LIVINGSTON and FREE (13) studied the behavior of the roots of some higher plants in relation to soils in which both the moisture content and the oxygen content were fairly well controlled. They found that the response of the plant to deficiency of oxygen in the soil atmosphere varied widely for different kinds of plants; some species (*Coleus blumei* and *Heliotropium peruvianum*, for example) were very sensitive to a deficiency

of oxygen in the soil atmosphere, while other species (for example, a species of *Salix*, probably *nigra*) gave no evidence of any injurious effects when the soil atmosphere was practically without oxygen. It was noted that cessation of intake of water by the roots, as detected by means of the porous-porcelain auto-irrigator (14), was the first sign of injury. With *Coleus* and *Heliotropium* this stopping of water absorption was followed by progressively lessened turgor of the shoot and leaf and finally by wilting and death. The plants could be revived when re-supplied with oxygen before wilting became too extensive or severe.

CANNON has made a prolonged and persistent study of the very difficult and no less important problem of the oxygen requirement of plant roots and has recently shown (4, 5) that, for roots growing in sand and with a maintained oxygen content of the soil atmosphere, the minimum oxygen content necessary for active root growth is much higher for higher temperatures than it is for lower temperatures, within the range from 18° to 30° C. This writer's comparisons between growth rates for different oxygen contents of the soil atmosphere are based upon what he terms the "normal" growth elongation rate for the maintained temperature employed. This rate is the one shown by roots of the kind in question growing at the given temperature, and under the non-oxygen conditions of his tests, when the oxygen content of the soil atmosphere is great enough so that any slight increase therein would not result in more rapid growth. In other words, the "normal" growth rate for any temperature is the maximum rate for that temperature and for the other non-oxygen conditions, both internal and external, that prevail in a test. It is clear that for any maintained temperature there must be a minimum oxygen partial pressure in the soil air, and a corresponding minimum in the oxygen-supplying power of the soil about these organs, for this elongation rate in any particular kind of roots. For roots that require atmospheric oxygen for growth there must also be a second minimum partial pressure of oxygen and rate of oxygen supply for any growth at all. For roots that need atmospheric oxygen, slow growth should occur with rates of oxygen supply slightly above the last named minimum, and progressively more rapid growth rates should be exhibited with correspondingly greater rates of oxygen supply until the first mentioned minimum is reached, after which higher rates of oxygen supply should give the growth rates called normal for the given temperature, etc., and for the roots in question.

Illustrations from CANNON's data are shown in table III, for *Zea mays* ("Golden Bantam" sugar corn) and for *Potentilla anserina*, a swamp plant.

From these data it is seen that the minimum oxygen content of the soil atmosphere for "normal" growth at 18° C. is five times as great for

TABLE III

RELATION OF TEMPERATURE AND SOIL-AIR OXYGEN CONTENT TO GROWTH RATE OF ROOTS

SOIL TEMPER- ATURE IN DEGREES C.	PERCENTAGE OF "NORMAL" GROWTH RATE				
	<i>Potentilla anserina</i>		<i>Zea mays</i>		
	1.2 per cent. oxygen in soil air	2.0 per cent. oxygen in soil air	3.0 per cent. oxygen in soil air	3.6 per cent. oxygen in soil air	10.0 per cent. oxygen in soil air
18	25	100	33	66	100
20	.		20
27	0	33
30		25	6	33	90

maize as it is for *Potentilla*, since the minimum oxygen supply for "normal" growth corresponds to a 10-per-cent. oxygen content in the soil atmosphere for maize and to a 2-per-cent. content for *Potentilla*. Also, the data show that, for the maintained temperatures studied, (between 18° and 30° C.), this minimum is greater for higher temperatures and smaller for lower ones.

CANNON's results suggest that the relation between root growth and oxygen supply to the roots may be generally found to be markedly influenced by the temperature of the soil (*i. e.*, of the roots) and also that the growth-temperature relations may prove to be correspondingly influenced by the oxygen-supplying power of the soil. Of course, it must be remembered that these relations are both surely influenced by many other kinds of conditions as well.

In his later publications CANNON (6, 7) has presented some evidence to the effect that the oxygen supply to roots of plants with aerial parts may, in some cases at least and to a considerable degree, depend upon downward movement of oxygen through the plant itself, from its aerial portions to the subterranean parts. It seems that this downward movement, which would constitute an *internal* oxygen supply to the roots, occurs much more rapidly during periods of photosynthetic activity in the leaves than in periods when the foliage is in darkness, and that it may sometimes be so rapid in periods of illumination of the leaves that oxygen may actually be given off from roots into the surrounding soil. If CANNON's as yet confessedly meager evidence is reliable, and there is every reason to suppose that it is, his suggestion that roots may in many instances secure an important supply of oxygen by the internal route, is an

exceedingly pregnant and important one for plant physiology, ecology, horticulture, agriculture and forestry. The present writer is not aware that this suggestion has ever before been given prominence. If correct, its importance can not be over-emphasized and it demands prompt and thorough investigation. Following this very valuable lead of CANNON's, it may be supposed: (1) that, in some or many instances, a greater or smaller part of the oxygen used by growing roots reaches them through the plant body itself, moving downward from the leaves, thus not being dependent directly upon the oxygen-supplying power of the root environment; (2) that another larger or smaller portion of the oxygen used by roots is absorbed from the soil, as in the case of the germinating seeds of the present studies; the magnitude of this portion being presumably dependent in general on the oxygen-supplying power of the soil; (3) that some of the oxygen absorbed from the soil by roots may have arrived there by previous outward movement from the same or other roots, especially during periods of foliar photosynthesis, with high partial pressure of oxygen in the illuminated green tissues; (4) that some of the oxygen moving downward through the plant may initially have entered the aerial parts from the air, via stomata, lenticels, etc.; and (5) that some of this descending oxygen may have been formed *de novo* by photosynthesis in the green leaves and, as molecular oxygen, may never have entered the plant at all. The last suggestion may be considered in connection with the downward movement of such substances as dextrose which has long been certainly known to move from green leaves to roots, though by just what means still remains uncertain. It may be that photosynthesis should receive emphasis as an oxygen-producing process occurring within the plant body, as well as being considered as a carbohydrate-producing process. And it may be that the mechanism of the descent of dextrose is the same or is related to the mechanism of the descent of oxygen.

The recent publications by CANNON, referred to in the preceding paragraph, present a large body of experimental data on the oxygen relations of roots, secured by new and ingenious methods, as well as much valuable discussion. They need not be further reviewed here, but readers interested in soil aeration in relation to root growth should refer to them. The results of CANNON's long-continued studies in this field seem to be generally consistent with those of the present experiments on seed germination described earlier in this paper, and it seems probable that the general principles of the oxygen relations for seed germination may generally or in many cases run parallel with those for the maintenance of health and growth in the roots of more mature plants, CANNON's suggestions about internally supplied oxygen being of course borne in mind. If it be assumed that the oxygen-supplying powers in CANNON's root environment

were approximately measured by the percentage of oxygen (partial pressure) in the soil atmosphere, it becomes possible to make a first and tentative comparison between that writer's results on the growth of maize roots and the results of the present studies on the germination of maize seed. With a temperature of 18° C. (not greatly different from that used in the germination experiments of the present studies) CANNON found that the minimum oxygen content (in the gas phase of the soil) that would allow good growth in maize roots was 10 per cent. Correspondingly, the minimum oxygen requirement for the germination of maize seed was found in the present studies to be between 3.0 and 2.3 mg. per square meter per hour. It is at any rate possible to state from CANNON's results that, with a temperature of about 18° C., the oxygen-supplying power necessary for good growth of his maize roots was presumably about five times as great as the corresponding requirement for his *Potentilla* roots. In the present studies seed-germination tests were not made with any seed having an oxygen requirement between that of maize and rice, but it seems probable that CANNON's *Potentilla* roots had an oxygen-supplying-power requirement more like that of germinating rice seed than like that of germinating maize seed. It will be remembered that rice seed germinates with an exceedingly low supply of oxygen.

It is perhaps unfortunate that the concept of the oxygen-supplying power of the soil has not previously attracted the attention that it clearly deserves and that earlier studies of soil aeration and of the relations of roots to soil oxygen have necessarily been based on considerations of the static partial pressure of oxygen in the gas phase of the soil. The relations between the percentage oxygen content of the soil air and the oxygen-supplying power of the soil need to be experimentally determined for a series of typical soils and for different water contents before quantitative comparisons may be attempted between the two kinds of measurements. It may be hoped that these relations will soon be studied by investigators in this very important province of physiological ecology. If it be true that the partial pressure of oxygen in the soil atmosphere may safely be considered as proportional to the oxygen supplying power of the soil, then that supposition surely requires experimental support. It seems likely, however, that these two soil characteristics may not be found to be generally proportional though they may well be so in such experimental procedures as those of CANNON. It should be emphasized that the carbon-dioxide-supplying power of the soil also greatly needs attention. It is desirable that the point of view and the method presented in the present paper be modified or replaced as far as logical and practical needs may be brought to light by further experience, and that natural and field soils be studied with respect to their oxygen-supplying powers and their carbon-

dioxide-supplying powers. It is also very desirable that experimental studies be undertaken with controlled conditions as well as for natural and field soils.

V. Summary and conclusions

The studies reported in the present paper have resulted in making available an exceedingly sensitive, practical method for comparing the oxygen-supplying powers of different environments. The method is based essentially on a colorimetric determination of the time required to produce a standard color change in an alkaline aqueous solution of pyrogallol, by the absorption of the oxygen brought to the indicator in a continuous stream of gas, this oxygen having been absorbed from the environment in question by a very thin-walled, water-impregnated, porous porcelain conical-shaped absorber that has capacity for absorbing oxygen and delivering it to the gas stream at least as rapidly as that element is supplied to the absorbing surface by the surroundings. The numerical results, called here indices of the environmental oxygen-supplying power, are expressed as milligrams of oxygen as supplied per hour through a square meter of absorbing area exposed to the environment in question.

During the development of the method a number of comparisons have been made with absorbers placed at different depths in soils of different moisture contents and degrees of packing. The results seem to establish clearly what has generally, but without direct evidence, been supposed to be true, namely, that the oxygen-supplying power of the soil environment is greater near the soil surface and for dryer and more loosely packed soils, while it is less at greater depths and for wetter and more firmly packed soils. With very wet, loamy garden soil the index value for a depth of 40 cm. may be only 1/140 (about 0.7 per cent.) as great as the corresponding value for a depth of 15 cm. Of course, for the same depth, moisture content, etc., the oxygen-supplying power may be expected to vary also with the kind of soil, whether sandy, loamy, clayey, etc., as well as with the prevalence of aerobic organisms and readily oxidizable materials in the soil between the absorber and the air-soil surface. Doubtless soil temperature also will be found to be influential, and barometric pressure and other climatic conditions may not be without influence. With wetting and partial drying of the soil, the index value for a given depth of a given soil fluctuates markedly, being generally smaller when the soil above the depth in question is wetter, and greater when it is dryer. It may be possible to classify field soils with regard to the relations obtaining between their oxygen-supplying powers and the climatic and crop conditions to which they are subject.

The apparatus and method devised for this work appear to be adequate for the experimental study of the dynamics of the soil-oxygen aspects of

ecology and agriculture. They should be valuable in many lines of research dealing with soil organisms as well as with plant roots. Also, the general principles involved should be valuable in studies of other environments besides soil environments, for other processes than vital ones and for some supplying powers other than that for elementary oxygen. Doubtless the method and apparatus may be improved in many ways, but the general principles involved seem to be clear. The definite comparisons between different environments, with respect to oxygen-supplying power, that now become possible should throw light on many problems in physiology, pathology, bacteriology, ecology, horticulture, agronomy, forestry, entomology, etc.

Some preliminary tests were made to gain an idea of the oxygen-supplying power requisite in an environment for the germination of several different kinds of seeds. Wheat germinates well, when well supplied with water and with temperatures about 22° C., in a soil with an oxygen-supplying power of 3.0 mg. or more per square meter per hour; while rice seed germinates well when the corresponding index value is as low as or lower than 0.5 mg. Wheat apparently failed to germinate at all with oxygen-supplying power rates below 1.5 mg. Rice germinated with the lowest supplying-power values tested in these studies. Each kind of seed appears to have its own minimum oxygen requirement but further studies will be necessary before seeds may be definitely classified on this basis.

These results regarding the oxygen-supplying-power requirement for seed germination are in accord with what might have been expected, that this requirement is not the same for all kinds of seeds, some seeds germinating well with exceedingly slow oxygen supply while others require a very rapid supply of that substance. The dynamic point of view and the quantitative method now introduced into the consideration of the oxygen need of germinating seeds may be valuable in the further advance of the physiology of seed germination and also in connection with germination problems encountered in ecology, agriculture, horticulture, forestry and related fields.

U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

LITERATURE CITED

1. ANDERSON, R. P. Reagents for use in gas analysis. I. Alkaline pyrogallol. *Jour. Ind. Eng. Chem.* **7**: 587-596. 1915.
2. ARNY, H. V., and RING, C. H. Standardized colored fluids. *Jour. Franklin Inst.* **180**: 199-213. 1915.
3. CANNON, W. A. Root-growth in relation to a deficiency of oxygen or an excess of carbon dioxide in the soil. *Carnegie Inst. Washington Yearbook* (1921) **20**: 48-51. 1922.

4. ————. The influence of the temperature of the soil on the relation of roots to oxygen. *Science* **58**: 331-332. 1923.
5. ————. Experimental investigations on roots. *Carnegie Inst. Washington Yearbook (1922-23)* **22**: 56-62. 1924.
6. ————. Experimental studies on roots. *Carnegie Inst. Washington Yearbook (1924-25)* **24**: 289-298. 1925.
7. ————, and FREE, E. E. Physiological features of roots, with especial reference to the relation of roots to aeration of the soil, by William Austin Cannon; with a chapter on differences between nitrogen and helium as inert gases in anaerobic experiments on plants, by Edward Elway Free. *Carnegie Inst. Washington Publ.* no. 368. 1925.
8. CLEMENTS, F. E. Aeration and air content; the rôle of oxygen in root activity. *Carnegie Inst. Washington Publ.* no. 315. 1921.
9. HARDY, F. Studies in West Indian soils. II. *West Indian Bull.* **19**: 189-213. 1922.
10. HOWARD, ALBERT. Recent investigations on soil-aeration. *Indian Forester* **44**: 187-212. 1918.
11. HUTCHINS, L. M., and LIVINGSTON, B. E. Oxygen-supplying power of the soil as indicated by color changes in alkaline pyrogallol solution. *Jour. Agr. Res.* **25**: 133-140. 1923.
12. LIVINGSTON, B. E. Paraffined wire pots for soil cultures. *Plant World* **9**: 62-66. 1906.
13. ————, and FREE, E. E. The effect of deficient soil oxygen on the roots of higher plants. *Johns Hopkins Univ. Circ. (n. s. 3)* **293**: 182-185. 1917.
14. ————. Porous clay cones for the auto-irrigation of potted plants. *Plant World* **21**: 202-208. 1918.
15. ———— and KOKETSU, R. The water-supplying power of the soil as related to the wilting of plants. *Soil Sci.* **9**: 469-485. 1920.
16. ————. Carbon-dioxide-supplying power of the air. *Carnegie Inst. Washington Yearbook (1921)* **20**: 71-72. 1922.
17. MASON, T. G. The water-balance of the plant and its significance in crop production. *West Indian Bull.* **18**: 157-184. 1921.
18. ROMELL, L. G. Luftväxlingen i marken som ekologisk faktor. *Meddel. Statens Skogsförsöksanst.* **19**: 125-359. 1922.
19. THONE, F. Ecological factors in region of Starved Rock, Illinois. *Bot. Gaz.* **74**: 345-368. 1922.
20. WHITNEY, M., and CAMERON, F. K. Investigations in soil fertility. *U. S. Dept. Agr., Bur. Soils Bull.* **23**. 1904.

NUTRITIONAL STUDIES ON *FUSARIUM LINI*¹

ERNEST SHAW REYNOLDS

(WITH THREE FIGURES)

I. Preliminary studies of some sugars and some nitrogen sources

The study of the nutritional relationships of parasitic fungi is fundamental to a satisfactory knowledge of the diseases which they produce as well as to a correct understanding of the causes of infection, parasitic invasion and resistance. The problem of resistance to disease is undoubtedly very complex, since not only are there many types of parasitic invasion, but there are also many contributing causes which interact with parasitic invasion to give rise to a given diseased condition. The nutritional demands of the invading organism must, however, be met by the host in order to have a successful invasion. Therefore a knowledge of the demands made by specific fungi, and of the substances available in their hosts, will lead to a better understanding of the conditions which make disease possible in those specific cases. Hence it is necessary to know the range of food substances of a given parasite and those available to it in a given host. Resistance as a *nutritional* problem, then, may be due either to the presence of antagonistic substances, or to the absence of essential food requirements, or to various combinations of these. The direct object of the present research is to learn the nutritional requirements and range of specific parasitic organisms, and, in specific hosts, the type, range and abundance of materials which may affect the growth of the parasite. The ultimate object is to give a more definite idea of the causes of resistance, especially where resistant and susceptible strains of the same host are known. It may well be expected also that careful attention to this subject will reveal many facts which will bring about a better understanding of the physiology of the higher plants. The physiological investigation of *obligate* parasites is difficult and the methods of attack upon the problems involved are vague and uncertain. It seems more hopeful to begin the study with organisms which are on the border line between saprophytism and parasitism and to progress toward the *obligate* parasites from that direction, building upon the knowledge gained from research on the facultative organisms. Such a program has been adopted in the physiological work of this department. A general survey of

¹ This is one of a series of investigations being conducted by the writer and his graduate students. The data upon which this paper is based were largely obtained by Mr. GEORGE C. MAYOUE, under the direction of the writer.

investigations upon fungous nutrition shows that relatively few intensive studies of a single parasitic organism have been made. The accumulation of information upon this subject is largely from rather scattered and more or less unrelated studies upon a number of organisms. It has seemed best, therefore, to attempt a rather complete investigation of one organism in order to correlate the various physiological facts with one another. In seeking an organism which would be appropriate for a continuous study our attention was naturally drawn to *Fusarium lini* Bolley, the causative organism of our common flax wilt disease. Since this fungus can be cultivated readily upon natural and artificial media, is rather widely tolerant of changes in its nutritional sources, and yet is known, up to the present time, only as a parasite upon flax, it seems well suited to the purpose already stated. Another feature which makes it of special interest is that certain strains or varieties of flax are very resistant to its attacks while others easily succumb. Moreover, the fact that it is of wide economic importance certainly does not lessen the advisability of making a careful study of its physiology.

REVIEW OF PREVIOUS WORK

The pathological examination of the flax wilt disease was largely carried on by BOLLEY (1) but no exact studies upon the nutrition of the fungus seem to have been published. TISDALE (4) in 1917 published an account of the inheritance and general nature of the disease in which a few nutritional questions received only incidental attention. The same investigator demonstrated that the flax wilt fungus will scarcely attack the host below 10° C. and thrives best at about 26–28° C. The most direct attack upon the problem of the nutrition of this fungus has been made by TOCHINAI (5). Several special sources of carbon were tried in comparison with one another for a period of two weeks. The amount of fungous growth was determined by weighing the dried mycelium. Representative carbohydrates, organic acids, higher alcohols and phenol derivatives were used as carbon sources. Inorganic and a few complex organic nitrogen compounds and amides were used as nitrogen sources. It was concluded that glucose and the disaccharides, sucrose and maltose, are the best carbon sources, while organic nitrogen was most available to this fungus.

TOCHINAI further studied various vegetable constituents as the chief source of food, but these gave no data as to the exact requirements of the fungus. He also used a synthetic medium with cane sugar and ammonium nitrate as the sources of carbon and nitrogen. The diversity of cultural conditions that could be used showed, however, the wide range of substances which this fungus may use as its source of nutrition. Conidium and chlamydospore formation varied somewhat with the type of medium employed.

The thick-walled spores were produced especially in those cultures which were unfavorable to mycelial formation.

PRELIMINARY SUGAR STUDIES

It seemed desirable as a foundation for later work to determine the amount of growth of *Fusarium lini*, which various sugars would produce. Fermi's medium, with the glycerine replaced by the various sugars, was adopted. Its composition is magnesium sulphate 2 grams, monopotassium phosphate 1 gram, dibasic ammonium phosphate 10 grams, and distilled water 1000 cc. The sugars² used are classified as follows: The pentose monosaccharides were represented by arabinose and xylose; the hexoses were levulose, d-galactose, glucose, and mannose. The two disaccharides were sucrose and maltose. For most sugars the concentrations used were M/5, M/10, M/15, and M/20 solutions; but M/4 was used additionally for the pentoses, M/40 for the disaccharides and M/25 for glucose.

Cultures were prepared so that each 125 cc. Erlenmeyer flask contained 40 cc. of the medium, and enough flasks were provided so that, for each sugar tested, two could be removed at the end of each week for the duration of the experiment. This means that for each sugar, fourteen flasks were usually prepared, although some sugar cultures were carried for ten or for fourteen weeks. The entire set was inoculated from *Fusarium lini* cultures which had been derived from a single spore isolation, and had also been tested for pathogenicity on flax plants in soil. The effort was made to inoculate the culture with equal quantities of mycelium and spores, although a preliminary experiment has shown that when cultures are carried as long as these the amount of inoculum makes little difference in the final maximum weight. The dry weight of fungus growth was determined for each culture and the results of each pair finally averaged. Check cultures of standard Fermi's medium in which glycerine was present were treated each week similarly to the sugar cultures.

The numerical results are summarized in table I.

It may be seen from these data that the nutritional value of the sugars is in the following descending order: glucose > maltose > sucrose > levulose > xylose > arabinose > mannose > galactose. The M/20 concentration of the disaccharides gave more fungous growth than the corresponding concentration of glucose, while in all higher concentrations glucose surpassed these other two sugars as a source of carbon supply. In the M/20 cultures

² The glucose, d-galactose, sucrose and maltose used were PFANSTIEHL sugars put out by the Special Chemicals Company. They are the most highly purified sugars obtainable and are essentially free from contaminating sugars or other carbohydrates (2). The xylose, arabinose and mannose were from the Digestive Ferments Company and the levulose from Merck and Co.

TABLE I
 DRY WEIGHT OF *F. lili* IN SUGAR CULTURES. AVERAGE DRY WEIGHT GIVEN IN GRAMS

CONCENTRATION OF SUGAR	WEEK	ARABINOSE	XYLOSE	GLUCOSE	MANNOSE	GALACTOSE	LEVULOSE	SUCROSE	MALTOSE	CHECK CULTURE
M/4	1	0.170	0.176							
	2	0.187	0.198							
	3	0.240	0.239							
	4	0.258	0.272							
	5	0.263	0.276							
	6	0.262	0.270							
	7	0.261	0.272							
M/5	1	0.074	0.098	0.183	0.183	0.170	0.169	0.123	0.169	0.0105
	2	0.104	0.169	0.295	0.194	0.176	0.187	0.187	0.194	0.0224
	3	0.211	0.219	0.379	0.209	0.206	0.229	0.286	0.300	0.0297
	4	0.198	0.241	0.404	0.234	0.219	0.282	0.305	0.366	0.0324
	5	0.188	0.269	0.433	0.226	0.215	0.294	0.319	0.390	0.0361
	6	0.172	0.262	0.464	0.215	0.216	0.291	0.316	0.400	0.0417
	7	0.170	0.214	0.473	0.220	0.216	0.285	0.312	0.404	
M/10	1	0.062	0.070	0.098	0.085	0.079	0.074	0.118	0.141	
	2	0.105	0.114	0.194	0.102	0.109	0.108	0.145	0.171	
	3	0.163	0.179	0.324	0.201	0.187	0.191	0.282	0.280	
	4	0.122	0.180	0.389	0.215	0.208	0.250	0.305	0.323	
	5	0.108	0.124	0.343	0.210	0.216	0.251	0.346	0.364	
	6	0.095	0.122	0.331	0.213	0.221	0.245	0.359	0.391	
	7	0.094	0.102	0.328	0.211	0.215	0.235	0.358	0.391	
M/15	1			0.088	0.062	0.049	0.040	0.024		
	2			0.175	0.082	0.101	0.094	0.091		
	3			0.213	0.141	0.186	0.169	0.181		
	4			0.284	0.187	0.198	0.194	0.198		
	5			0.298	0.192	0.197	0.197	0.208		
	6			0.290	0.188	0.194	0.196	0.210		
	7			0.281	0.181	0.198	0.192	0.215		
M/20	1			0.027	0.008	0.0095	0.009	0.073	0.092	
	2			0.092	0.010	0.0109	0.0118	0.114	0.134	
	3			0.122	0.015	0.0165	0.0190	0.177	0.198	
	4			0.129	0.0135	0.0131	0.0154	0.203	0.219	
	5			0.084	0.013	0.0131	0.0138	0.214	0.216	
	6			0.071	0.0125	0.0125	0.0136	0.218	0.219	
	7			0.062	0.0116	0.0121	0.0134	0.215	0.222	

the maltose and sucrose are essentially of the same value as the M/10 cultures of the monosaccharides mannose and galactose. This seems to indicate that in these dilute concentrations of the disaccharides, the carbon is as available to the fungus as it is in mannose and galactose, since the carbon in the M/10 monosaccharides is quantitatively about the same as that in the M/20 disaccharide media. In more concentrated solutions the disaccharides are somewhat better carbon sources than these two hexoses. Maltose almost uniformly gave a better growth than sucrose, possibly due to the fact that when it breaks down it gives rise to two glucose molecules while the hydrolysis of sucrose gives one of glucose and one of levulose. The latter is a less valuable carbon source than the glucose. The carbon value of the pentoses relative to the other sugars must be determined in the light of the fact that they contain one less carbon atom per molecule than the hexoses. A culture containing M/4 concentration of a pentose contains somewhat more carbon than a solution of M/5 hexose. In cultures of M/4 concentration using arabinose and xylose, there was somewhat greater growth than in the M/5 galactose and mannose. It would seem therefore that these four sugars are about equal as carbon sources for this fungus when equalized as to the amount of carbon present. Possibly the carbon of the pentoses is a little more available than it is in the two hexoses, galactose and mannose. The glycerine of the check cultures gave uniformly poorer growth than all but the lowest concentrations of sugars, showing that at least in this type of medium it is notably inefficient as a carbon source. These results agree very well with the conclusions of TOCHINAI in so far as there is a duplication of work.

It is interesting to note that WEIMER and HARTER (7) obtained only 0.1094 grams of growth of *Fusarium acuminatum* on a 10 per cent. glucose medium in two weeks, while nearly all of these sugars in much weaker solution, about 3.5 per cent., gave a better growth of *Fusarium lini* in the same period.

PRELIMINARY NITROGEN STUDIES

In another series of cultures of *Fusarium lini* carried for two months the ammonium phosphate of Fermi's medium was replaced by various nitrogen sources. Each nitrogen source was so calculated that the same quantity of nitrogen was present in all cultures. Ten culture tubes of each medium were prepared and the combined dry weight of growth was determined at the end of two months.

All cultures were inoculated with the same strain of fungus. After two months the growth was washed and dried on an oven-dried and weighed filter paper. The net dry weight of each culture is given in grams in table II.

TABLE II
GROWTH OF *Fusarium lini* WITH VARIOUS SOURCES OF NITROGEN

NITROGEN SOURCE	DRY WEIGHT IN GRAMS
Potassium nitrate	1.5133
Calcium nitrate	1.1551
Ammonium lactate	1.1273
Ammonium phosphate, dibasic	1.0233
Asparagin	0.8711
Potassium nitrite	0.6649
Ammonium sulphate	0.3933
Urea	0.2189
Ammonium lactate without glycerine	0.1267
Calcium nitrite	none

From the data it is seen that potassium nitrate, calcium nitrate, ammonium lactate and ammonium phosphate provide the best sources of nitrogen for this fungus under the conditions of the experiment, and in the order named. Asparagin, potassium nitrite, ammonium sulphate and urea are next best in the order given, while calcium nitrite gave no growth. Ammonium lactate did not seem to supply the amount of carbon necessary for satisfactory growth when it was the sole source of both nitrogen and carbon.

TOCHINAI used the same *percentage* of all nitrogen sources in his cultures, thus supplying the fungus with different amounts of nitrogen in the different cultures. In the series here reported the various nitrogen compounds were calculated to supply the same amount of nitrogen in each culture. The results show that, contrary to the conclusions of TOCHINAI, the best sources of nitrogen are not organic materials, but the nitrates of potassium and calcium, with the ammonium salts of lactic and phosphoric acids rather close seconds. This series did not include the indefinite material, peptone, which TOCHINAI included and which in the concentration used was the best source of nitrogen. Both investigations demonstrated that nitrites are poor sources of nitrogen for this fungus.

A culture medium supplying its carbon in the form of glucose and its nitrogen in the form of potassium nitrate would seem to be the best synthetic type for *Fusarium lini* so far discovered.

II. Effects of flax extracts on *Fusarium lini*

As a contribution to the solution of the problem of the cause of resistance of certain strains of flax to the wilt disease, a series of experiments was planned to determine what effects extracts of flax plants would have upon the growth of *Fusarium lini* in pure culture.

The flax plants used were of two strains, one which will be called "susceptible" easily succumbs to the wilt disease in "wilt sick soil" while the second, called "resistant," was of the strain known as NDR No. 114, one of the most resistant types developed by Professor H. L. BOLLEY. The plants were gathered when about eight inches tall, dried rapidly on racks

TABLE III

GROWTH OF *Fusarium lini* IN FERMI'S MEDIUM IN PRESENCE OF EXTRACTS OF RESISTANT AND SUSCEPTIBLE FLAX. DRY WEIGHT OF MYCELIUM IN GRAMS

TREATMENT OF MEDIUM	TIME IN WEEKS	PERCENTAGE AND KIND OF FLAX EXTRACT USED								
		Check	Two per cent.		Four per cent.		Six per cent.		Ten per cent.	
			R*	S*	R	S	R	S	R	S
Series A										
Glycerine not autoclaved, extract autoclaved.	1	0.0110	0.0143	0.0146	0.0210	0.0224	0.0217	0.0229	0.0343	0.0346
	2	0.0219	0.0214	0.0215	0.0269	0.0297	0.0289	0.0291	0.0365	0.0359
	3	0.0307	0.0312	0.0321	0.0333	0.0347	0.0328	0.0359	0.0391	0.0466
	4	0.0319	0.0343	0.0350	0.0349	0.0390	0.0385	0.0403	0.0402	0.0474
	5	0.0360	0.0389	0.0393	0.0398	0.0420	0.0396	0.0434	0.0409	0.0479
	6	0.0401	0.0374	0.0395	0.0393	0.0431	0.0394	0.0450	0.0424	0.0490
Series B										
Glycerine not autoclaved, extract through filter cylinder.	1	0.0110	0.0094	0.0101	0.0031	0.0085	0.0080	0.0091	0.0028	0.0036
	2	0.0219	0.0173	0.0191	0.0050	0.0094	0.0090	0.0111	0.0048	0.0081
	3	0.0307	0.0229	0.0246	0.0096	0.0097	0.0109	0.0131	0.0075	0.0148
	4	0.0319	0.0294	0.0293	0.0130	0.0147	0.0093	0.0139	0.0060	0.0159
	5	0.0360	0.0309	0.0324	0.0196	0.0208	0.0078	0.0136	0.0055	0.0152
	6	0.0401	0.0319	0.0336	0.0200	0.0218	0.0068	0.0130	0.0052	0.0141
Series C										
Glycerine autoclaved, extract autoclaved.	1	0.0106	0.0136	0.0133	0.0204	0.0191	0.0209	0.0216	0.0332	0.0347
	2	0.0209	0.0214	0.0211	0.0266	0.0266	0.0284	0.0289	0.0345	0.0353
	3	0.0294	0.0313	0.0311	0.0334	0.0340	0.0329	0.0371	0.0381	0.0390
	4	0.0319	0.0340	0.0398	0.0337	0.0400	0.0383	0.0392	0.0390	0.0401
	5	0.0363	0.0384	0.0485	0.0392	0.0404	0.0393	0.0396	0.0399	0.0418
	6	0.0416	0.0389	0.0498	0.0384	0.0414	0.0388	0.0402	0.0389	0.0430
Series D										
Glycerine autoclaved, extract through filter cylinder.	1	0.0106	0.0090	0.0096	0.0030	0.0042	0.0075	0.0085	0.0024	0.0036
	2	0.0209	0.0170	0.0174	0.0043	0.0072	0.0048	0.0087	0.0041	0.0050
	3	0.0294	0.0213	0.0224	0.0084	0.0099	0.0078	0.0198	0.0057	0.0069
	4	0.0319	0.0286	0.0305	0.0126	0.0138	0.0092	0.0209	0.0050	0.0072
	5	0.0363	0.0295	0.0318	0.0184	0.0190	0.0063	0.0185	0.0051	0.0077
	6	0.0416	0.0287	0.0329	0.0193	0.0202	0.0054	0.0180	0.0049	0.0066

* R = resistant; S = susceptible.

during the summer of 1922, and stored in boxes until used. They were then ground in a mill and the powder was steeped in water about 48 hours. The liquid was filtered off through glass wool and then through filter paper. This filtrate was sterilized by passing it through a Mandler diatomaceous filter into sterile flasks in which it was stored until needed. Measured amounts of the sterile flax extract were added to Fermi's solution, in such proportions that 40 cc. of medium had the same concentration as the standard medium, and the percentage of flax extract varied as indicated in table III, 2, 4, 6 and 10 per cent. being used. All cultures were inoculated with essentially the same amount of *Fusarium lini*. Two cultures were removed from each set at the end of each week of growth for six weeks, and the entire contents of each was placed on an oven-dried, weighed filter paper and washed with 100 cc. of water. The fungous growth was then oven-dried at 85-90° C. on the filter paper in a weighing glass. The net weights of fungous growth are used as an indication of the amount of growth, under the stated conditions.

Four series of cultures with resistant flax extract were run, two with the glycerine of the medium added before autoclaving, and two with the glycerine added after autoclaving. Four corresponding series with susceptible flax extract were run.

EFFECTS OF AUTOCLAVING FLAX EXTRACT VERSUS STERILIZATION BY DIATOMACEOUS FILTER

In series A and C (table III) the flax extract was autoclaved. It will be seen from fig. 1 that in all the cultures in which autoclaving of the extract occurred there was an evident increase in growth over the check cultures which received no flax extract. It is clear also that the higher percentages, autoclaved, consistently provided a better medium throughout the whole period of growth than either the check or the lower percentages. It will be seen, however, that in a considerable number of cases there was actually a smaller weight of growth at the end of the sixth week than at the end of the fifth (figs. 1, 2 and 3). This did not occur in the check cultures, and in fact no decrease was recorded in them until the tenth week.

In series B and D, table III, the flax extract was sterilized through a filter cylinder and in general the opposite growth relations existed. The ten per cent. extract proved to be a poorer medium than any of the lower concentrations, as shown in fig. 2. All of the various concentrations supported less growth than the check cultures for the corresponding growth periods.

From these observations it is evident that the process of autoclaving caused some profound modification of the flax extract which changed it from a depressor of growth for *Fusarium lini* to an accelerator of its growth.

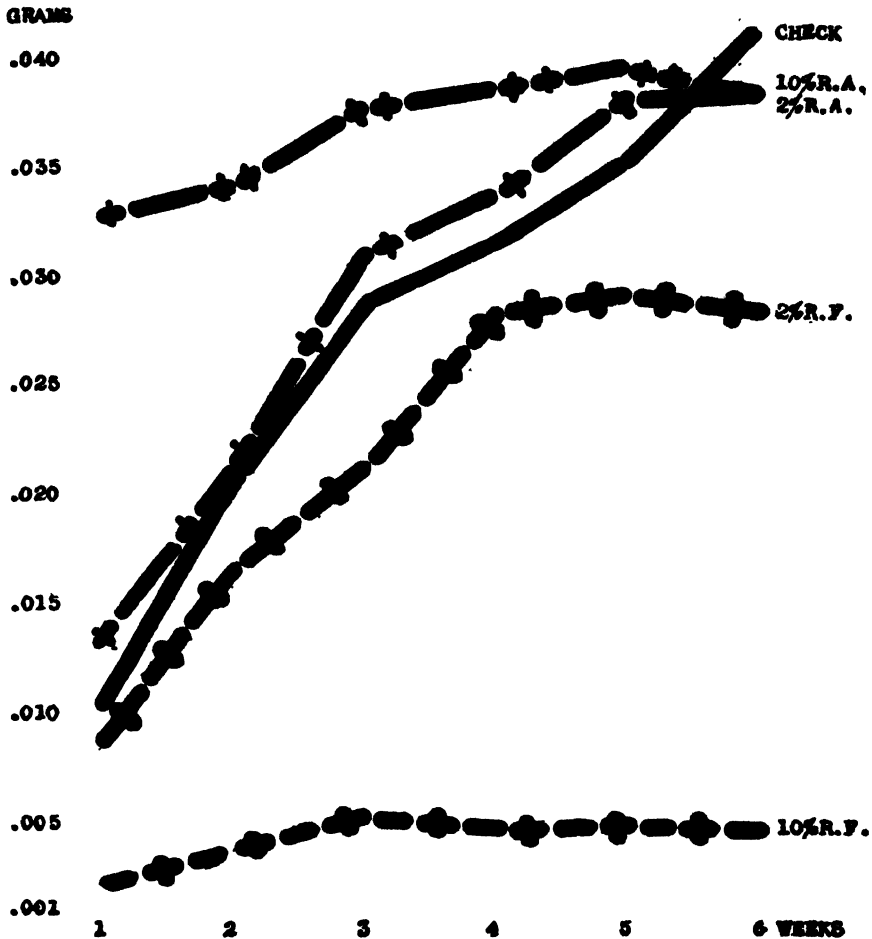


FIG. 1. Dry weight growth curves of *F. lini*. Medium with 2 or 10 per cent. resistant-flax extract (R). Extract autoclaved (A), or filter sterilized and unheated (F). Control culture (check), without flax extract.

This could be caused by several possible changes, such as the destruction of an injurious material, or of an enzyme which was in some manner effective in depressing growth. Or, substances of doubtful food value might be changed to some more available form. That the natural flax extract contains injurious materials is evident from the reduced growth of all cultures on the filter-sterilized extract in series B and D. These are shown, as compared with the check cultures, and with those on the autoclaved extract in fig. 3. The original medium in each case contained the same amount of

nutritive material as the check, but upon the addition of flax extract further foods were added. This is evident from the fact that the autoclaved media gave greater growth than the check.

It is known that flax plants contain a glucoside which is decomposed by an enzyme, producing hydrocyanic acid. This latter substance has been shown by REYNOLDS (3) to be a strong inhibitor of growth for *Fusarium lini*. In the extract which was sterilized in the autoclave the enzyme must have been destroyed and the hydrocyanic acid itself was probably largely driven off, while any food materials contained in the extract were added to those in Fermi's medium (fig. 1). Thus growth of the fungus would be

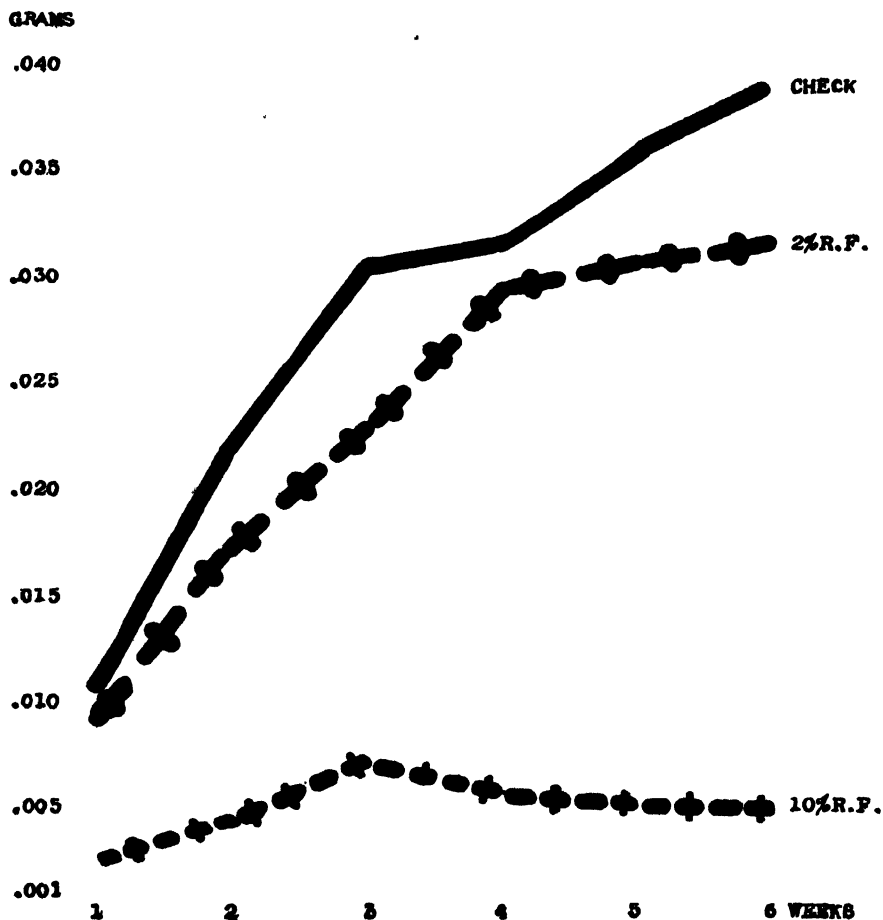


FIG. 2. Dry weight growth curves of *F. lini* on medium with 2 or 10 per cent. of resistant-flax extract (R). Extract filter sterilized and unheated. Control culture (check), without flax extract.

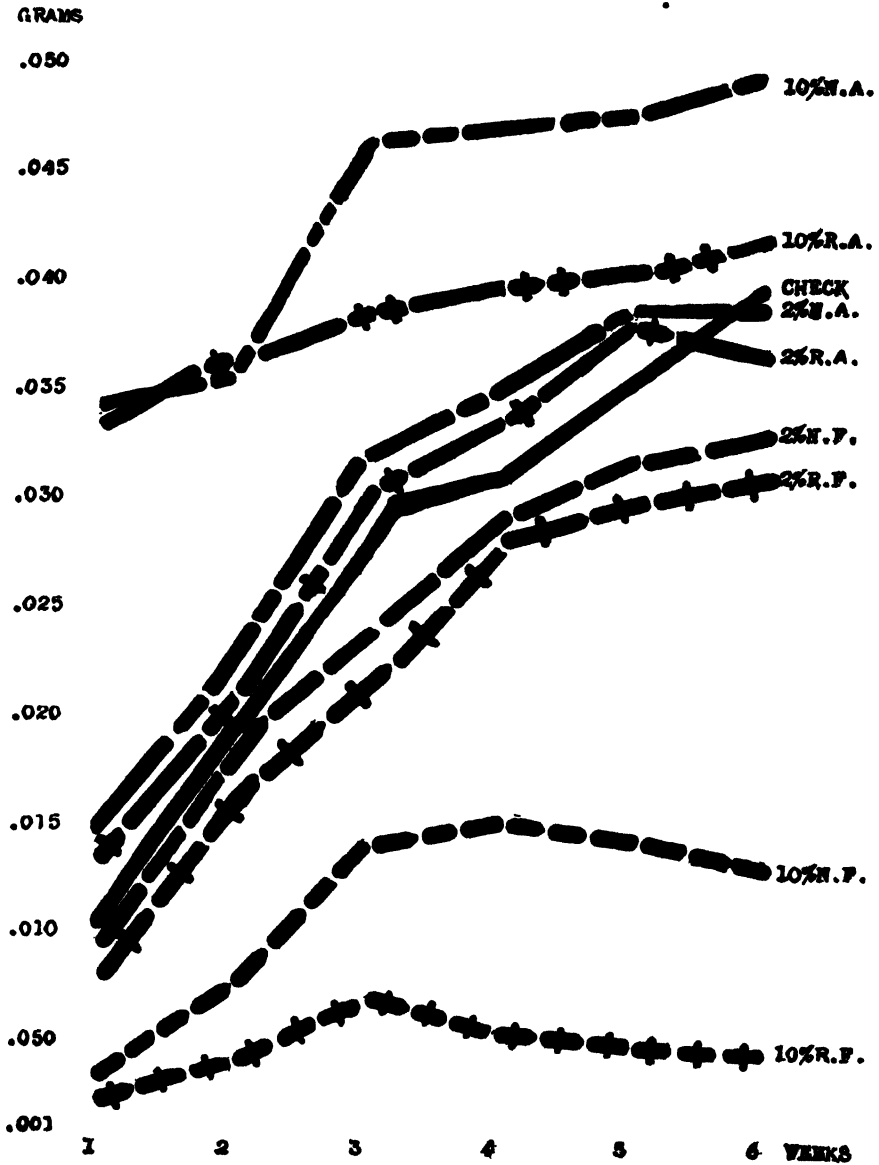


FIG. 3. Dry weight growth curves of *F. lini*. Medium with 2 or 10 per cent. of resistant-flax extract (R), or of nonresistant flax (N). Extract autoclaved (A), or filter sterilized and unheated (F). Control cultures (check), without flax extract.

stimulated in the autoclaved extract roughly in proportion to the amount of extract added. Most, if not all, of the glucoside must have been hydrolyzed during the time of steeping the flax powder, and an unpublished series of experiments shows that autoclaving may drive off a considerable amount of the hydrocyanic acid in a culture medium. It appears possible, therefore, that the inhibitive substance in the cultures of series B and D was the hydrocyanic acid which was derived from hydrolysis of the glucoside by the enzyme naturally present, while in series A and C the hydrocyanic acid was largely driven off by autoclaving.

EXTRACT FROM RESISTANT AND SUSCEPTIBLE FLAX IN RELATION TO GROWTH OF *Fusarium lini*

A comparison of the effects of the extracts from the resistant and susceptible strains of flax upon the growth of *Fusarium lini* shows an interesting phenomenon. Almost uniformly the extract from the susceptible flax gave a better growth of fungus than that from the resistant flax (fig. 3). This would show that the factor for depressing growth of the fungus is present in greater quantity in the resistant-flax extract than in that from the susceptible flax. Were the increased growth due only to larger quantities of food materials in the extract from the susceptible strain of flax, we should not find a smaller growth in any case than in the check cultures. However, in all cases in series B and D, the filtered extract, the growth was less than in the corresponding check cultures, though greater with the susceptible than with the resistant extract of equal concentration.

DETERMINATION OF HYDROCYANIC ACID IN FLAX EXTRACTS .

As a step in the analysis of the problem of resistance in the flax it was necessary to determine the presence and amount of hydrocyanic acid in the flax extract. The hydrolysis of the glucoside during the 48 hours maceration resulted in the formation of prussic acid, which was demonstrated by a method involving the precipitation of silver cyanide in a standard solution of silver nitrate.

Flax of both the resistant and susceptible varieties was finely ground and equal weights of the powder were steeped for 48 hours in distilled water in Kjeldahl flasks. Then a slow distillation was carried on, passing the distillate into a standardized solution of silver nitrate which converted the hydrocyanic acid contained in the distillate into silver cyanide. After the distillation was fairly advanced phosphoric acid was added and the distillation continued to dryness. One hundred cc. quantities of the distillate were then titrated by a solution of potassium cyanide of known concentration to determine the amount of silver nitrate not combined with the hydrocyanic

acid of the distillate. This titration was carried out in a test-tube. The precipitate produced by adding the potassium cyanide is at first fine, giving a milky appearance to the whole tube. A rather violent agitation, however, flocculates the precipitate and clears the solution so that an end point can be determined by the cessation of the appearance of the milky precipitate. Verification of the end point is attained by filtering and testing one portion of the filtrate with a few drops of silver nitrate and another portion with potassium cyanide. A lack of precipitation in either case verifies the end point.

One hundred cc. of distillate from the resistant flax required 7.8 cc. of the KCN solution to combine with the excess silver nitrate in the distillate. The same quantity of distillate from the non-resistant flax required 9.4 cc. of the KCN solution. Since the same quantity of silver nitrate solution was originally present in the distillate it is evident that the resistant flax extract produced more hydrocyanic acid than the non-resistant flax extract.

Discussion

The four series of cultures reported above, together with other unpublished data, seem to give a reasonable basis for an hypothesis concerning the cause of resistance to the flax wilt disease shown by certain strains of flax. Since the extract from resistant flax, when passed through the filter cylinder, depressed growth of the flax wilt fungus more than the extract from the susceptible variety, it would seem to indicate that the cause of resistance in this strain is a chemical condition. Since the same types of extracts, when autoclaved, accelerated growth, it appears that the factor causing depression of growth is a relatively labile or volatile compound. It is known that in the young flax plant the glucoside linamarin, or phaseolunatin, is present, and that the juices of crushed flax plants change this into glucose and the volatile hydrocyanic acid.

The determination of the relative quantity of prussic acid in the two flax extracts demonstrates that the resistant strain contains more glucoside than the non-resistant. A possible explanation of resistance is that the flax wilt fungus in invading a resistant flax plant with a relatively high quantity of linamarin might easily release sufficient hydrocyanic acid to inhibit its own growth either completely or partially, while in the more susceptible strains there may not be a sufficient production of the poison to prevent the firm establishment of the fungus.³ Observations have shown that resistance in flax is a relative quality and not absolute, which would be explained by

³ Since the completion of this work the interesting paper on onion smudge by WALKER (6) and his co-workers has appeared. It is possible that the non-volatile toxic material of colored onions may be related to a cyanogenetic glucoside from which HCN may be released by enzymic action in a manner similar to that suggested for flax wilt.

the facts brought out elsewhere by the writer (3), showing that the fungus is able to grow in cultures that contain the equivalent of at least a .03M concentration of KCN, but is largely prevented from growing in concentrations much higher. Experiments are planned, or under way, to determine the use which *Fusarium lini* may be able to make of various glucosides, the distribution of linamarin in the flax plant, the conditions under which the glucoside is most abundant in the flax, and studies of similar character upon other resistant varieties. It is to be determined also just what effects the flax extracts will have upon the growth of *Fusarium lini* in media which are especially favorable to it. It is expected also to ascertain how other *Fusaria* and also other parasites of flax are affected by the flax extracts.

AGRICULTURAL COLLEGE,
NORTH DAKOTA

LITERATURE CITED

1. BOLLEY, H. L. Flax wilt and flax sick soil. N. D. Agr. Exp. Sta. Bull. 50: 1-58. 1901.
2. PFANSTIEHL, CARL, and BLACK, ROBERT S. The rare sugars: Their purity and tests. Jour. Ind. Eng. Chem. 13: 685-687. 1921.
3. REYNOLDS, E. S. Some relations of *Fusarium lini* and potassium cyanide. Amer. Jour. Bot. 11: 215-217. 1924.
4. TISDALE, W. H. Flaxwilt: A study of the nature and inheritance of wilt resistance. Jour. Agr. Res. 11: 573-605. Plates 44-46. 1917.
5. TOCHINAI, Y. Studies on the food relations of *Fusarium lini*. Am. Phytopath. Soc. Japan 1: 22-33. 1920.
6. WALKER, J. C., LINDGREN, CARL C., and BACHMANN, FRED A. Further studies on the toxicity of juice extracted from succulent onion scales. Jour. Agr. Res. 30: 175-187. 1925.
7. WEIMER, J. L., and HARTER, L. L. Glucose as a source of carbon for certain sweet potato storage rot fungi. Jour. Agr. Res. 21: 189-210. 1921.

ADSORPTION AS A MEANS OF DETERMINING RELATIVE HARDINESS IN THE APPLE

STUART DUNN AND A. L. BAKKE

Introduction

Hardiness in the apple excites the greatest consideration when one is selecting varieties for an orchard in a northern latitude or in a region with a rigorous climate. The only definite procedure to ascertain this has been to grow the apple tree to bearing age. The underlying reasons why one variety of apple will maintain itself under climatic extremes of drought, heat and cold, while another will not, have been sought since pioneer days. The problem is of particular economic importance in the upper Mississippi valley because there are practically no commercial orchards of winter apples of any kind in this region. Orchardists, nursery men and fruit breeders, unmindful of the size of their task and the time factor involved, have attempted to secure by "trial and error" methods a knowledge of the nature of the conditions producing the qualities they sought. Considering the proposition from any point of view it would seem that a hardy variety must have some constant characteristic or combination of characteristics by which it may be distinguished from varieties which are tender, very early in life, and thus shorten the time and lessen the labor of selection in apple breeding.

In considering a problem of this kind it should be borne in mind that the units of which plant tissue is composed are the cells and it is within these individual cells that the factor or combination of factors which make for hardiness reside. These cellular units may be regarded as colloidal systems, the greater part of which are hydrophilic in nature. It would only be natural to consider that in these colloidal materials are to be found the forces which make for hardiness.

Historical Review

As early as 1860 SACHS (33) stated that ice formation in plant tissue takes place principally in the intercellular spaces, and hence rupture of the cells may not be directly attributed to freezing. MÜLLER-THURGAU (22, 23) concluded from his experiments that freezing of plant tissue is accompanied by a withdrawal of water from the cell and the formation of ice crystals in the intercellular spaces. On thawing, this water of the inter-

cellular spaces escapes by transpiration, causing the plant to wilt; the killing of the cell may be due to a loss of water. Mez (19) on the other hand held that death is due in the case of each particular plant to a fatal "minimum" temperature rather than to injury through direct desiccation.

The results obtained by WIEGAND (38, 39, 40) in 1906 are in close agreement with those of MÜLLER-THURGAU. He studied microscopically the effect of low temperature on buds of the horse chestnut and other trees. He was able to detect ice in the intercellular spaces when the temperature had fallen as low as -18°C . Water probably came out of the cell through the equalizing action of the force of imbibition following the abstraction of water by the ice crystals from the surface film lining the intercellular spaces. He concluded that in no case can the death of any plant be traced directly to absolute cold alone at a temperature below the freezing point. There is a critical point in the moisture content of every cell, beyond which death results due to the actual withdrawal of water to form ice, not to shock, overstimulation, nor any other action of cold. A hardy plant holds water with such tenacity that not enough can be extracted by cold to cause death. Similar conclusions were drawn by WHIPPLE (37) in observing winter injury to apple and pear trees in Montana. The work of UPHOF (36) on cold resistance in spineless cacti gives results agreeing with those of WIEGAND and MÜLLER-THURGAU.

GORKE (13) observed an increase in salt concentration of the cell sap as a result of freezing. He advanced the idea that frost injury was due to the precipitation of proteins through salting out. The effect of the increased concentration of acid salts was considered to be small, however, and insufficient to account for the precipitation. In this statement he was supported by LIDFORSS (18), who investigated some of the winter green flora of Sweden. It was found that these plants had in their leaves large amounts of sugar and some oil, but no starch. He proposed the theory that this sugar had a protective action in retarding the salting out of the proteins. BLACKMAN (5) pointed out that this theory does not hold in the case of the sugar beet and sugar cane, both of which have a high sugar content but at the same time could not be considered as being in any way resistant to low temperature.

One of the most extensive contributions to this subject is that of CHANDLER (9) on the killing of plant tissue by low temperature. Basing his reasoning on the theory of MÜLLER-THURGAU he formulated the idea that some plants might be hardy because the plasma membrane has the property of withstanding great loss of water and that this property might be due, in part at least, to a high sap density or solute content. The force of imbibition which he admits may be the most important is here disregarded. He determined the sap density of a large number of different

plants by using the freezing point depression method. The salt content of the cells was increased by watering the plants with solutions of salts of varying amounts, and distilled water was used on the control plants. His results uniformly showed that the killing temperature was reduced whenever the sap density of the tissue was increased. This apparently shows that the sap density is correlated with hardiness in the succulent plants here studied, but in a later publication (8) involving leaves and twigs of apple and peach trees this was not found to hold true. Sap from these tissues was studied to find the relation of its various physical and chemical properties such as freezing point lowering, molecular concentration, and electrolytic conductivity to winter hardiness. No changes, however, could be found that would give any information as to the nature of the change by which plants acquire maturity, or the greater resistance to cold to be observed in winter. Similar conclusions were made by LEWIS and TUTTLE (17) who investigated the osmotic properties of leaves of some evergreens at Ontario, Canada. The osmotic pressure of the cell sap was in no case great enough to account for prevention of freezing of tissue.

HARVEY (14) made extensive studies on the nature of the hardening process in succulent plants such as cabbage and tomato and found that the principal effect of the hardening process is a change in the constituents of the protoplasm which prevent their precipitation as a result of the physical changes incident upon freezing. The proteins are changed to forms less easily precipitated as indicated by an increase in the amino-acid content of the cabbage plants on hardening. The factors which produce protein precipitation on the freezing of a plant juice are held to be principally the increase in the hydrogen-ion concentration and the increase in the concentration of the salts. The latter factor was held to be insufficient to cause precipitation except under the conditions of a changed acidity. NEWTON (24), on the other hand, in an investigation of the hardiness of winter wheat, found no constant relation between the hydrogen-ion concentration, depression of the freezing point, or specific conductivity of the cell sap and relative frost hardiness. He also reported that the relation between water soluble nitrogen and hardiness was not constant.

Working on the question of the dormancy in the plum STRAUSBAUGH (35) found that the water in the tissue of hardy trees is held more firmly or in a more stable state than in the tender ones. Microchemical studies showed that dormancy involved a protoplasmic change in the buds that was much more pronounced in the hardy varieties. Protein was evidently modified but just how was not known. He stated that it is possible that there is induced, as a result of these modifications, a very decided change in the colloidal condition within the cells which increases the force of imbibition so that the water of the protoplast is retained against the dehydrating force of freezing.

BEACH and ALLEN (4) studied the structure and composition of twigs of numerous varieties of apples to find if these qualities correlate with hardiness. Cutting, compression and penetration tests, as well as specific gravity tests, seemed to indicate some correlation between hardness of wood and hardiness but exceptions were found. Maturity of the wood and low moisture content at the beginning of cold weather is stressed as an important factor. They concluded that from the practical viewpoint it is impossible to name any one test by which the degree of constitutional hardiness of a seedling apple may be foretold. Maturity of tissue as a factor in hardiness was also emphasized by GLADWIN (12), SHUTT (34), ROBERTS (29), POTTER (28), CARRICK (7), BRADFORD (6), HOWARD (16), and DORSEY and BUSHNELL (10).

The physico-chemical properties of the sap of seventeen apple varieties were tested by BAKKE, RADSPINNER, and MANEY (3). Since it appeared that the freezing point lowering difference for different varieties was not in itself sufficient to indicate differences in hardiness these investigators made a series of tests at five different times of the year, not only of the freezing point depression, but of the water content, ash content, and hydrogen-ion concentration as well. The results showed considerable variation, although some correlations to hardiness were found.

HOOKE (15) compared hardy and non-hardy apples as to pentosan content of the twigs. Excellent correlation was found between the development of pentosans and resistance to low temperatures. A correlation was also present between pentosan content and water holding and absorbing capacity as measured in a sulfuric-acid-filled desiccator. He advanced the theory that the pentosans, or perhaps some specific pentosan, function in the plant tissue by holding water, which is in the nature of adsorbed or colloidal water, and that this water cannot freeze when the plant is subjected to ordinary winter conditions. ROSA (30, 31, 32), working on vegetable plants such as cabbage, cauliflower, lettuce, and tomato, got results similar to those of HOOKER. There was a marked parallel between pentosan content and hardiness, indicating that pentosans may be related in part causally to cold resistance. He concluded that salt content, acidity, hydrogen-ion concentration, sugar, moisture, and protoplasmic colloids other than pentosans are also partly responsible.

Among the more recent contributions on hardiness are two by NEWTON (24, 25). Both of them deal with frost resistance in winter wheat. He worked with several varieties of winter wheat which could be divided into three groups: very hardy, medium, and tender. He determined several properties of the hardened leaf tissue to discover any possible correlation. These properties were depression of the freezing point, specific conductivity, hydrogen-ion concentration of the cell sap, dry matter content, water

soluble nitrogen, and sugar content. No constant relation between any of them and hardness could be found. However, he did find that the imbibition pressure of fresh leaves in the winter hardened condition was in most cases directly related to hardness. Pressures were as high, in some cases, as 600 atmospheres. The volume of press juice obtained per 100 grams of hardened leaves was inversely proportional to the hardness of a variety. Unhardened leaves showed neither of these properties. The imbibition pressure of hardened leaves appeared to depend on the physical state of the cell colloids characteristic of living tissues, since this property was lost when the tissue was killed. The quantity of hydrophilic colloids in the press juice of hardened tissues was found to be directly proportional to hardness.

It is recognized that the colloids found in a growing plant are in competition with the colloids in the soil medium. This is particularly evident in the case of water absorption. To determine the amount of colloidal content present in soils the adsorption of certain dyes from solutions by the soil was found to be a reliable test for colloidal content.

PEARCE and MILLER (27) worked on adsorption of dyes by glacial clays. The clays studied were negatively charged in their colloidal form, and therefore should markedly adsorb such positive dyes as methylene blue and should show very little attraction for the negative dye eosin. Such was found to be the case, the adsorption of eosin being relatively slight and therefore somewhat irregular. The dye solutions were shaken with samples of clay weighing one gram or less and then allowed to stand until settled. Equal amounts of the supernatant dye solution were drawn off and the relative color intensity read with a Duboseq colorimeter.

Three different methods were used by ANDERSON, FRY, GILE, MIDDLETON, and ROBINSON (1) to estimate the amount of colloidal material in soil samples through adsorption. These were (a) water vapor, (b) ammonia and (c) the dye, malachite green. For this last test a solution of the dye was added to the soil sample (0.25 to 1.0 gram), shaken continuously for one hour, and then centrifuged to throw down the suspended matter. The supernatant liquid was then drawn off and the amount of dye remaining determined by reading with a Duboseq colorimeter against a standard dye solution. It was calculated from the adsorption of mineral powders and the mineralogical composition of soils that as a rule less than five per cent. of the total adsorption of the soil is due to the non-colloidal part. Adsorption by non-colloidal constituents should, therefore, not seriously affect adsorptive methods for determining the amount of colloids in soils. In another publication (11) these same workers carried out further tests of colloidity by adsorption of malachite green. The results agreed very closely with tests on adsorption of ammonia gas and of water vapor.

ASHLEY (2) in testing clays for plasticity used the dye adsorption method for determining colloidal matter. Brilliant green, malachite green, and gentian violet were some of the dyes used. Malachite green seemed most suitable. He found that the adsorption of a dye by clays supplies an approximate measure of plasticity. MOORE, FRY, and MIDDLETON (21) also used malachite green and secured very nearly the same results as when a dry gas is adsorbed by dry colloidal substance. This fact in itself would seem to justify confidence in the results. PATTEN and WAGGAMAN (26) used gentian violet quite extensively. This dye was found to be well adapted for studying distribution of solute between soil and solution because it was changed very slightly by contact with soils used and because it can be readily estimated colorimetrically to within a fraction of a part in a million of solution.

WILKINSON and HOFF (41) experimented on the adsorptive power of soils using methylene blue, diamine blue 3B, and neutral violet. MILLER (20) in studies on adsorption by activated sugar charcoal was able to show that soils and charcoal have in common the property of removing from solution many of the same types of electrolytes such as the basic dyes, malachite green and methylene blue, acids, salts, etc. The dyes seemed to be held in much the same way by these adsorbents.

It appeared that similar tests applied to plant tissue might also indicate its colloidal content and such results might correlate with observed hardness. It was with this end in view that these investigations were undertaken.

Materials and methods

The material used in this work was taken from twigs of twenty-two different varieties of apples grown in the Seion Orchard of the Mount Arbor Nursery at Shenandoah, Iowa. They were all well known commercial varieties possessing a graduation in resistance to unfavorable weather conditions from extremely hardy down to the rather tender. In general they might be arranged into three groups. Among the more hardy are such varieties as Hiberna, Patten Greening, McIntosh, Oldenberg or Duchess, Wealthy, Virginia Crab, and Red Astrachan. At the other extreme among the markedly tender ones are Ben Davis, Stayman, Winesap, Baldwin, Delicious, Maiden Blush and Winter Banana. Somewhere between these will be grouped those medium in hardness, such as Malinda, Fameuse, Peerless, Salome, Roman Stem, Northwestern Greening, and Yellow Transparent.

The twigs were all gathered November 11, 1924, from trees that were growing under as nearly the same conditions as possible. Maturity was at hand as the growing season was ended. The twigs were ground rather coarsely in a power food chopper at once, then carefully air dried, after

which they were ground more finely in a disk power mill. The material was sifted through a 100 mesh sieve. Nearly all that would pass through was found to be composed largely of cambium, cortical and other non-woody tissue. That remaining was woody tissue very easily distinguished because of its fibrous nature. For purposes of brevity, the material which sifted through will be here spoken of as cortical and the other as non-cortical. Since the region thus designated as cortical is the seat of most of the growth of the twig it may thus be regarded as the most vital part. It was thought to be the logical tissue on which to make tests for ability to withstand winter killing. These investigations were therefore centered on this tissue.

In these tests such dyes as methylene blue, acid fuchsin, eosin, gentian violet, and malachite green were used. Stock solutions of the dyes were made up at a strength of 0.1 gram of dye to 100 cc. distilled water and these were diluted in varying amounts for different tests. For the tests with eosin the stock solution was diluted 1 to 10. A sample of tissue weighing 0.5 gram was placed in a test bottle and to it was added 25 cc. of dilute dye solution. It was shaken at frequent intervals for one hour and then placed in a centrifuge and whirled for five minutes at a speed of 1,000 revolutions per minute. The bottle was then removed and the clear supernatant liquid decanted into one of the cups of a Duboseq colorimeter. In the other cup was placed some of the pure dye solution and the amount of dye removed by the sample found by comparison.

Results

In the case of eosin several samples indicated no adsorption by the material. Acid fuchsin also gave no adsorption indicating that the material is negatively charged like the soils studied by PEARCE and MILLER (27). Consequently no further tests were made with these dyes.

In working with methylene blue, several dilutions of the stock solution were tried and the one most practicable was found to be 1 to 32, that is, a match of colors in the colorimeter was most easily obtained at this dilution. This dye showed a very marked adsorption by all of the samples. Since these tests are only comparative, one of the most convenient ways of expressing the results is by the fraction of dye left in solution after treating the sample, which is obtained by dividing the reading of the standard by the reading of the solution tested. The results of this series of tests appear in table I arranged in the order of their numerical magnitude.

By this arrangement there are five varieties that do not conform to the general arrangement of approximate hardiness. These are Northwestern Greening, Hiberna, Wealthy, Oldenberg, and Patten Greening. The rest, although not grouped exactly in the order of known hardiness, yet arrange themselves in three general groups, hardy, semi-hardy, and tender.

TABLE I

ADSORPTION OF METHYLENE BLUE BY PULVERIZED TISSUES OF APPLE TWIGS

VARIETY	A SAMPLE READING	B STANDARD READING	B/A FRACTION OF DYE LEFT IN SOLUTION
Red Astrachan	37.0	0.7	.019
McIntosh	27.2	0.6	.022
Northwestern Greening	29.1	0.8	.027
Virginia Crab	25.2	0.7	.029
Peerless	17.8	0.5	.028
Salome	22.9	0.8	.035
Yellow Transparent	28.5	1.0	.035
Jonathan	21.2	0.8	.038
Malinda	25.4	1.0	.039
Stayman	33.3	1.4	.042
Roman Stem	26.2	1.2	.046
Hibernal	23.7	1.1	.046
Wealthy	29.1	1.4	.048
Winesap	13.6	0.7	.051
Winter Banana	27.1	1.4	.052
Oldenberg	21.7	1.4	.064
Ben Davis	20.6	1.4	.068
Patten Greening	14.6	1.0	.068
Delicious	11.2	1.0	.089
Fameuse	22.0	1.6	.092
Maiden Blush	45.1	4.4	.098
Baldwin	33.3	3.9	.117

Gentian violet was then tried in varying dilutions and with varying hydration periods. In some series of tests the samples were allowed to stand as long as over night in the dye solution in order to determine whether the time of hydration might be a factor. No better correlation to hardness than the results given in the first table was found.

Lastly, malachite green was used. As with the other dyes a stock solution was made up at the rate of 0.1 gram of dye to 100 cc. distilled water. Samples of material weighing 0.5 gram were likewise used. Several series of tests with varying dilutions of the stock solution and varying hydration periods from one hour to several hours were made but in no case was the correlation any better than before.

With the previous tests the material was allowed to remain in the dye solution for a considerable length of time, usually at least an hour and in some series of tests as long as over night. It might be supposed, then, that this length of time might allow considerable alteration both of the dye itself and of the colloidal matter. Acting on this hypothesis in an effort to secure

a better correlation a series of tests was made in which the time of treatment was greatly shortened. The stock solution of malachite green was diluted 1 to 15 for this series. The half gram sample and 25 cc. of the dye solution were shaken for 15 seconds and centrifuged for 10 minutes after which comparison was at once made in the colorimeter. The results of this short time test appear in table II.

TABLE II

ADSORPTION OF MALACHITE GREEN BY PULVERIZED TISSUES OF APPLE TWIGS

VARIETY	A SAMPLE READING	B STANDARD READING	B/A FRACTION OF DYE LEFT IN SOLUTION
Peerless	43.2	2.0	.0463
Wealthy	42.0	2.0	.0577
Hibernal	46.6	2.3	.0501
Red Astrachan	42.8	2.3	.0537
Virginia Crab	23.5	1.3	.0554
McIntosh	44.5	2.5	.0562
Oldenberg	42.0	2.4	.0572
Maiden Blush	36.8	2.3	.0625
Patten Greening	41.5	2.9	.0652
Yellow Transparent	44.5	3.0	.0675
Malinda	40.0	3.0	.0750
Salome	40.0	3.0	.0750
Roman Stem	39.5	3.0	.0760
Stayman	35.0	2.7	.0771
Winter Banana	41.2	3.2	.0777
Fameuse	42.0	3.4	.0809
Jonathan	35.6	3.0	.0843
Northwestern Greening	43.7	3.2	.0951
Baldwin	41.0	5.0	.1220
Winesap	40.0	5.0	.1250
Delicious	40.0	5.1	.1270
Ben Davis	40.0	5.5	.1380

Here it will be noticed that the correlation to hardness is much better. Only about three or four varieties are noticeably placed differently from the hardness grouping, notably the Maiden Blush, Stayman and Fameuse. Fameuse is usually regarded as rather hardy and Stayman and Maiden Blush as tender.

Early in these investigations it was found difficult to obtain a good match of the colors when comparing them in the colorimeter due to coloring matter dissolved from the tissue itself and which usually imparted a brownish or yellowish tinge to the solution. An effort was made to counteract

this by use of amber glass color screens but this was not entirely satisfactory. Finally the scheme was tried of treating 0.5 gram samples of tissue with distilled water only and using the resulting colored solution as a color screen. This was done for each variety and it was suspended in a vial beneath the cup containing the standard by means of a wire. By using this form of compensator excellent results were obtained. It was possible to obtain a much better match of colors than before. The results of a series of tests with malachite green at a dilution of the stock solution of 1 to 10 appear in table III.

TABLE III

ADSORPTION OF MALACHITE GREEN BY PULVERIZED TISSUES OF APPLE TWIGS

VARIETY	A SAMPLE READING	B STANDARD READING	B/A FRACTION OF DYE LEFT IN SOLUTION
Patten Greening	43.2	1.0	.0232
Yellow Transparent	35.0	0.9	.0257
Oldenberg	35.0	1.0	.0285
Virginia Crab	28.0	0.8	.0285
McIntosh	39.8	1.2	.0302
Hibernal	41.6	1.3	.0312
Peerless	48.0	1.7	.0354
Red Astrachan	47.5	1.7	.0358
Wealthy	37.0	1.4	.0378
Malinda	44.9	1.8	.0401
Northwestern Greening	38.3	1.6	.0417
Salome	42.8	1.9	.0444
Fameuse	43.0	2.0	.0465
Jonathan	34.4	1.6	.0465
Roman Stem	42.0	2.0	.0476
Maiden Blush	37.0	1.8	.0476
Winter Banana	30.0	1.5	.0500
Winesap	41.5	2.1	.0506
Stayman	41.0	2.2	.0536
Baldwin	41.0	2.3	.0561
Delicious	41.0	3.0	.0733
Ben Davis	40.2	3.7	.0920

Here it will be noted that the varieties are grouped very nearly in the order of actual hardness, indicating that the adsorptive powers of the colloids present are correlated with hardness. Experienced orchardists, nurserymen, and horticulturists disagree as to the exact relative position of some of these varieties but in general the grouping will be much as given in the above table, the most hardy first.

Discussion and conclusions

It should be pointed out here that there is a remarkable similarity between the adsorptive behavior of this plant tissue, and that exhibited by the soils and charcoal studied by MILLER (20) and the soils studied by PEARCE and MILLER (27), ASHLEY (2), and others. These materials seem to have in common the property of removing from solution many of the same types of electrolytes such as the basic dyes, malachite green and methylene blue, and show no adsorption with acid dyes. It is well known that the amount of dye these materials will remove from solution has been used as a measure of the adsorptive capacity of decolorizing charcoals and of the clay or colloidal constituents of soils. The dyes appear to be held in much the same way by these adsorbents as by the plant tissues here tested. After a dye has been removed from aqueous solution by either of these three substances, it may be recovered unaltered from the adsorption complex, in part at least, by simple extraction with alcohol, acetone, or other organic solvents. It is interesting and perhaps significant that such apparently different substances should have so many of these properties in common. Soils and this pulverized plant tissue are very complex materials which afford possibilities for many types of reactions, while pure charcoal, which is ordinarily considered to be very inert chemically, can give rise to only one type of reaction and that by virtue of the forces existing at its surface.

The results of these tests indicate rather conclusively that the hydrophilic colloids of the plant are in a large measure concerned in hardness. In this they agree very closely with the results of NEWTON (25), HOOKER (15), ROSA (32), and others, whose work on colloidal matter of plant tissues has shown a close correlation between the quantity of such material present, and hardness. That is, it appears certain that the hydrophilic colloids by reason of their relatively enormous adsorptive surfaces hold water within the cell and prevent death from the dehydrating force of freezing. Furthermore, these results indicate that this property may be quantitatively measured in a convenient manner by the adsorption of aniline dyes from solution.

In the tests that have been made it is clear that these measurements of adsorption may be conveniently and advantageously used in measuring the relative hardness of different varieties of the apple.

LITERATURE CITED

1. ANDERSON, M. S., FRY, W. H., GILE, P. L., MIDDLETON, H. E., and ROBINSON, W. O. Adsorption by colloidal and non-colloidal soil constituents. U. S. Dept. Agr. Bull. **1122**: 1922.
2. ASHLEY, H. E. The colloidal matter of clay and its measurement. U. S. Geol. Survey Bull. **388**: 1909.
3. BAKKE, A. L., RADSPINNER, W. A., and MANEY, T. J. A new factor in the determination of the hardness of the apple. Proc. Amer. Soc. Hort. Sci. **1920**: 279-289.
4. BEACH, S. A., and ALLEN, F. W., JR. Hardiness in the apple as correlated with structure and composition. Iowa Agr. Exp. Sta. Res. Bull. **21**: 1915.
5. BLACKMAN, F. F. Vegetation and frost. New Phyt. **8**: 354-363. 1909.
6. BRADFORD, F. C. The relation of temperature to blossoming in the apple and the peach. Missouri Agr. Exp. Sta. Bull. **53**: 1922.
7. CARRICK, D. B. Resistance of the roots of some fruit species to low temperatures. Cornell Univ. Agr. Exp. Sta. Memoir **36**: 1920.
8. CHANDLER, W. H. Sap studies with horticultural plants. Missouri Agr. Exp. Sta. Res. Bull. **14**: 1914.
9. ———. The killing of plant tissue by low temperature. Missouri Agr. Exp. Sta. Res. Bull. **8**: 1913.
10. DORSEY, M. J., and BUSHNELL, J. W. The hardness problem. Proc. Amer. Soc. Hort. Sci. **1920**: 210-224.
11. GILE, P. L., MIDDLETON, H. E., ROBINSON, W. O., FRY, W. H., and ANDERSON, M. S. Estimation of colloidal material in soils by adsorption. U. S. Dept. Agr. Bull. **1193**: 1924.
12. GLADWIN, F. E. Winter injury of grapes. New York (Geneva) Agr. Exp. Sta. Bull. **433**: 1917.
13. GORKE, H. Über chemische Vorgänge beim Erfrieren der Pflanzen. Landw. Vers. Stat. **65**: 149-160. 1906.
14. HARVEY, R. B. Hardening process in plants and development from frost injury. Jour. Agr. Res. **15**: 83-112. 1918.
15. HOOKER, H. D. Pentosan content in relation to winter hardness. Proc. Amer. Soc. Hort. Sci. **1920**: 204-207.
16. HOWARD, R. F. The relation of low temperature to root injury of the apple. Nebraska Agr. Exp. Sta. Bull. **199**: 1924.
17. LEWIS, F. J., and TUTTLE, G. M. Osmotic properties of some plant cells at low temperatures. Ann. Botany **34**: 405-416. 1920.
18. LIDFORSS, B. Die wintergrüne Flora. Eine biologische Untersuchung. (Abstract.) Bot. Centbl. **110**: 291-293. 1909. Original, Lunds Univ. Arsskr. n. f. Bd. 2 afd. 2 no. **13**: 76 pp. 1907.

19. MEZ, C. Einige pflanzengeographische Folgerungen aus einer neuen Theorie über das erfrieren Eis-beständiger Pflanzen. *Bot. Jahrb.* **34** (Beibl. 79): 40-42. 1905.
20. MILLER, E. J. Adsorption by activated sugar charcoal. *Michigan Agr. Exp. Sta. Techn. Bull.* **73**: 1925.
21. MOORE, C. J., FRY, W. H., and MIDDLETON, H. E. Methods for determining the amount of colloidal material in soils. *Jour. Ind. Eng. Chem.* **13**: 527-531. 1921.
22. MÜLLER-THURGAU, H. Über das Gefrieren und Erfrieren der Pflanzen. *Landw. Jahrb.* **9**: 133-189. 1880.
23. ———. Über das Gefrieren und Erfrieren der Pflanzen. II. *Landw. Jahrb.* **15**: 453-610. 1886.
24. NEWTON, ROBERT. A comparative study of winter wheat varieties with especial reference to winter killing. *Jour. Agr. Sci.* **12**: 1-9. 1922.
25. ———. The nature and practical measurement of frost resistance in winter wheat. *Univ. of Alberta College of Agr. Res. Bull.* **1**: 1924.
26. PATTEN, H. E., and WAGGAMAN, W. H. Absorption by soils. *U. S. Dept. Agr. Bur. Soils Bull.* **52**: 1908.
27. PEARCE, J. N., and MILLER, L. B. The colloidal properties of pleistocene clays. *Jour. Phys. Chem.* **26**: 1-24. 1922.
28. POTTER, G. F. Experiments on resistance of apple roots to low temperatures. *New Hampshire Agr. Exp. Sta. Techn. Bull.* **27**: 1924.
29. ROBERTS, R. H. The development and winter injury of cherry blossom buds. *Wisconsin Agr. Exp. Sta. Res. Bull.* **52**: 1922.
30. ROSA, J. T., JR. Investigations on the hardening process in vegetable plants. *Missouri Agr. Exp. Sta. Bull.* **48**: 1921.
31. ———. Nature of hardening in vegetable plants. *Proc. Amer. Soc. Hort. Sci.* **1919**: 190-197.
32. ———. Pentosan content in relation to hardness of vegetable plants. *Proc. Amer. Soc. Hort. Sci.* **1920**: 207-210.
33. SACHS, J. Bericht über die physiologische Thätigkeit an der Versuchstation in Tharandt. *Landw. Vers. Stat.* **2**: 167-201. 1860.
34. SHUTT, F. T. On the relation of moisture content to hardness in apple twigs. *Trans. Roy. Soc. Can. Ser. 2.* **9** (Sec. 4): 149-153. 1903.
35. STRAUSBAUGH, P. D. Dormancy and hardness in the plum. *Bot. Gaz.* **71**: 337-357. 1921.
36. UPHOF, J. C. TH. Cold resistance in spineless cacti. *Arizona Agr. Exp. Sta. Bull.* **79**: 1916.

37. WHIPPLE, O. B. Winter injury to fruit buds of the apple and the pear. *Montana Agr. Exp. Sta. Bull.* **91**: 1912.
38. WIEGAND, K. M. Some studies regarding the biology of buds and twigs in winter. *Bot. Gaz.* **41**: 373-424. 1906.
39. ————. The occurrence of ice in plant tissue. *Plant World* **9**: 25-39. 1906.
40. ————. The passage of water from the plant cell during freezing. *Plant World* **9**: 107-118. 1906.
41. WILKINSON, J. A., and HOFF, WILBER. Adsorption of dyes by soils. *Jour. Phys. Chem.* **29**: 808-815. 1925.

A STUDY OF THE CLEARING OF ALCOHOLIC PLANT EXTRACTS*

W. E. LOOMIS

Clearing is generally a necessary part of the analytical procedure with plant materials preserved and extracted with 80 per cent. alcohol, as advocated in a previous paper (3). Clearing is equally necessary when samples, killed and preserved by drying, are not given a preliminary extraction with ether before extracting with alcohol. The work reported here suggests that this necessity is based more upon the mechanical removal of lipoids, waxes, and certain colloids not precipitated by 80 per cent. alcohol, than upon the chemical removal of copper reducing substances. It is probable that comparable, although somewhat high, reducing-sugar values may be obtained with certain alcoholic plant extracts without either preliminary ether extraction or clearing. On the other hand, the alcoholic extract from fresh mangel leaves so clogs the filter, when evaporated and taken up in water, as to be unfilterable by ordinary means. The same is true of a large number of leaf, green-fruit, and woody-stem extracts.

Correspondence with a number of physiological chemists indicated a lack of uniformity in clearing methods. A number of men were using neutral and basic lead acetate interchangeably although the work of BRYAN (1) indicates the danger of such a procedure. With these conditions in mind we have attempted to determine the limits of value and safety in the use of neutral- and basic-lead-acetate solutions for clearing plant extracts.

General procedure

Stock extracts of plant tissues, so chosen as to give a considerable range of clearing conditions, were prepared from fresh green material of known moisture content by dropping the sample into enough boiling 95 per cent. alcohol to give a final concentration of 80 per cent. After boiling for 2 or 3 minutes the samples were sealed and set aside 24 hours or longer. The extract was then decanted off, 2 ml. of 80 per cent. alcohol added for each gram of fresh material used, and the extraction repeated. The extracts were combined and preserved in glass containers. Samples of 100 to 300 ml. were taken for clearing and reduction, depending upon the reducing power of the extract. These were placed in pyrex beakers and the alcohol

* The work reported here was done at CORNELL UNIVERSITY during the tenure of a NATIONAL RESEARCH COUNCIL fellowship in the biological sciences.

removed on a boiling water bath. Evaporating 200 ml. of a celery extract to 20 ml. when strong alcohol fumes were still given off gave a value of 28.0 mgs. dextrose per sample. Evaporating to 2 ml. which completely removed the alcohol gave a value of 29.0 mgs. dextrose. Since the difference of 1 mg. is within the error of weighing, the practice of evaporating to 3 to 5 ml. was adopted. One hundred milliliters of distilled water, less the quantity to be added in the lead solution, were added and heated to 80° C. to soften gummy precipitates and insure the solution of all reducing substances. As a further precaution the sides of the beaker were rubbed with a rubber police and insoluble masses were finely divided. After cooling to room temperature the desired quantity of lead solution was added and the samples filtered immediately into 200 ml. beakers to which had been added an estimated excess of potassium-oxalate crystals.¹

The lead precipitate was allowed to drain on the filter and was washed with cold water until the filtrate no longer gave a precipitate in the oxalate solution below. The presence of an excess of oxalate was assured by testing with a drop of dilute lead-acetate solution. The delead solution, which was kept within a volume of 160 ml., was then filtered into a 200 ml. graduated flask, beaker and precipitate carefully washed, and the solution adjusted to 200 ml. at 20° C. in a water bath. Three 50 ml. portions were taken for copper reduction by the QUISUMBING and THOMAS procedure (6). Copper was determined by direct weighing of the cuprous-oxide precipitate. Several samples from each lot were retained and the purity of the precipitate determined by the official, sulphuric-nitric-acid, electrolytic method (5). Copper was deposited on a revolving platinum grid cathode by a current of 2 amperes for a period of 55 minutes. Calculations are based on the tables by QUISUMBING and THOMAS.

It was found practicable to hold the weights of the three copper precipitates to a maximum variation of 0.0015 gms. on the lighter and 0.0020 gms. on the heavier samples. This limits the error of any figure included in the data to three-tenths of a milligram of dextrose and requires differences of one to two milligrams for significance.

Experimental results

CONDITIONS AFFECTING THE DESTRUCTION OF REDUCING SUBSTANCES BY LEAD COMPOUNDS

When the preliminary experiments indicated considerable loss in the reducing activity of plant extracts treated with excesses of basic lead acetate,

¹ Potassium oxalate was chosen as a deleading reagent on the basis of the work of SAWYER (7), EYNON and LANE (2), MEAD and HARRIS (4) and experimental data to be presented in a later paper. We have found potassium oxalate both safe and convenient when used in excess and the precipitate allowed to crystalize before filtration. The practice of filtering the cleared solution onto the oxalate crystals allows both of these conditions to be met.

it became important to determine the conditions under which these losses could be reduced to a minimum. One milliliter of 1.25 sp. gr. lead-acetate

TABLE I

THE RELATIONS OF TIME AND TEMPERATURE TO THE DESTRUCTION OF REDUCING SUBSTANCES BY LEAD COMPOUNDS

SOLUTIONS CLEARED WITH 5 TIMES THE QUANTITY OF LEAD NECESSARY TO GIVE A SLIGHT EXCESS AND DELEADED WITH POTASSIUM OXALATE AS INDICATED	REDUCING ACTIVITY OF MANGEL-LEAF EXTRACT AS A PERCENTAGE OF CHECK (1 ML. NEUTRAL)	
	Cleared with neutral lead acetate	Cleared with basic acetate
Deleaded at once at room temperature..	99.3	86.5
Heated for 15 minutes after deleading	93.9	78.6
Heated for 15 minutes before deleading	71.5	47.5
Left at room temperature for 14 hours before deleading	88.6	77.6

solution was sufficient to give a slight excess with the extract used for this experiment. Five milliliters were accordingly used for the comparisons. The data in table I indicate that increasing the quantity of neutral lead acetate used in clearing to 500 per cent. of the check had no effect on the reducing activity of the extract when the lead was removed at once. Basic lead gave a significant loss under the same conditions. Any standing before deleading, or heating with or without deleading (potassium oxalate does not remove all the lead), lowered the reducing activity, but the percentage reduction was always greater when basic lead was used.

A COMPARISON OF NEUTRAL AND BASIC CLEARING

The reducing values of a number of plant extracts cleared with minimum and excess quantities of neutral- and basic-lead-acetate solutions are compared in table II. The minimum dose of lead was taken as the quantity of 1.25 sp. gr. solution which gave a distinct, white precipitate when a drop of dilute potassium-oxalate solution was added to the extract-lead solution mixture. With the samples used, this varied between 1 and 2 ml. of solution. Five times the minimum dose was taken for the excess treatment. For ready comparison the reducing value of the minimum or low-neutral clearing is given in milligrams dextrose and the other treatments are calculated as a percentage of this value. Percentage differences which may be considered to be significant vary from 4 or 5 per cent. with the small reducing values to 1 or 2 per cent. with the larger. The data given in table

II show that, with the exception of the apple-wood extract, an excess of neutral lead acetate which was five times the quantity required for clearing had no effect upon the reducing power of the solutions. The minimum dose of basic lead acetate lowered the reducing power an average of 3 per cent. and this was increased to 11 per cent. by the excess treatment. In addition the variation between best and poorest recovery was about twelve times greater with the excess-basic than it was with the excess-neutral clearing. While a comparison of the absolute quantities of reducing substances in sweet potato roots and mangel leaves will rarely be required, that method which will most nearly give such values is most likely to give reliable results when comparing sweet potato roots of varying composition.

Although an excess of neutral lead decreased the reducing power of apple-wood extract this reduction was apparently due to an incomplete precipitation of reducing impurities when 2 ml. of lead solution were used. When 4 ml. was taken as the check value, high neutral gave a value of 98.4 per cent. which compares with the values obtained with the other extracts. The apple-wood extracts gave a very heavy precipitate with lead acetate and the first formation of a precipitate with potassium-oxalate solution appar-

TABLE II

A COMPARISON OF THE REDUCING POWER OF EXTRACTS CLEARED WITH NEUTRAL AND BASIC LEAD ACETATE SOLUTIONS

CLEARING TREATMENT	LOW NEUTRAL AS MGS. DEXTROSE, OTHER TREATMENTS AS A PERCENTAGE OF THIS VALUE							
	Mangel leaf extract	Tomato leaf no. 1	Tomato leaf no. 2	Sweet potato roots	Apple wood no. 1	Apple wood no. 2	Celery stalks	Spinach leaves
Low neutral ..	25.5	26.1	40.2	56.3	43.2	97.6	154.2	17.4
Excess neutral	98.8	99.6	99.8	98.6	94.9	94.0	100.0	..
Low basic	97.7	98.9	99.3	96.8	96.1	92.7	98.3	..
Excess basic ..	79.6	85.4	87.8	96.3	85.9	87.4	93.5	88.5

ently did not indicate sufficient excess of neutral lead to complete the removal of active impurities.

COMPLETENESS OF CLEARING.—When a small but distinct excess of neutral lead acetate was used the color of the solutions so cleared compared favorably with that of the best clearing obtained. Low basic was almost invariably less satisfactory than either of the neutral clearings from the standpoint of color and ease of handling. High basic was normally lightest in color, but showed a distinct tendency to darken if allowed to stand after

deleading. Water-clear solutions were obtained with all of the extracts used, by deleading with hydrogen sulphide after adding an excess of neutral lead acetate. Although hydrogen sulphide left the solutions acid to methyl red while they were boiled to expel the excess of the gas, such a treatment gave very close checks with potassium-oxalate deleading on an unhydrolyzed celery extract which contained approximately twice as much non-reducing as reducing sugars. Neutral-lead-acetate-hydrogen-sulphide clearing is recommended for trial when complete removal of coloring matter is required. It has no advantage when reducing sugars are to be estimated with Fehling's solution, and is less convenient. The precipitation of a large excess of lead with hydrogen sulphide will lower the reducing power of the solution, presumably by co-precipitation of reducing substances.

NEUTRAL AND BASIC CLEARING OF PURE REDUCING-SUGAR SOLUTIONS.—Extensive tests were made of the effect of neutral and basic lead acetate on the reducing power of pure sugar solutions and on the purity of the reduced cuprous oxide. The data given in table III are typical of the results obtained and agree with those presented in table II. The low-neutral clearing with potassium-oxalate deleading, which was used as a standard in this work, is shown to give reliable results on pure sugars. Also, a large excess of neutral lead acetate is much less destructive of reducing sugars than is a corresponding excess of basic acetate. There is less difference in the stability of the two sugars than would have been expected from the work of BRYAN (1). The same results were obtained, however, when known quantities of levulose and dextrose were added to plant extracts of known reducing value. When 22 mgs. of dextrose were added to an apple-wood extract, 98.2 per cent. was recovered from the high-neutral and 90.4 per cent. from the high-basic clearing. Of 18 mgs. of

TABLE III

THE EFFECT OF CLEARING ON PURE SUGAR SOLUTIONS

TREATMENT SOLUTIONS CLEARED AND DE- LEADED AS ABOVE—1 AND 5 ML. OF THE LEAD SO- LUTIONS WERE ADDED	REDUCING SUGAR FOUND—CALCULATED AS MGS. AND AS PER CENT. OF CHECKS (UNCLEANED)			
	LEVULOSE		DEXTROSE	
	Mgs. sugar found	Recovery as per cent. of check	Mgs. sugar found	Recovery as per cent. of check
Pure solutions (check)	182.9	132.2
Low-neutral clearing	183.0	100.06	132.1	99.92
High-neutral clearing	182.4	99.73	130.8	98.94
Low-basic clearing	179.3	98.03	131.3	99.32
High-basic clearing	159.5	87.21	122.5	92.66

levulose added to parallel samples, 96.1 per cent. was recovered from the high-neutral and 86.6 per cent. from the high-basic clearing. A third series of extracts receiving the same clearing treatments was used as the basis of comparison.

A STUDY OF THE LOSS OF REDUCING SUBSTANCES IN BASIC CLEARING

The consistently lower reducing value of solutions cleared with basic lead may be assumed to be due to one of the following causes or to a combination of them: (1) The loss may be due to the co-precipitation or reducing substances. (2) It may be due to the formation of an insoluble, sugar-lead compound or to the formation of a slightly soluble compound which is co-precipitated with, or adsorbed by the clearing precipitates. Or (3), it may be due to the removal of organic substances which are precipitated and weighed as cuprous oxide when clearing with neutral lead acetate. The last supposition is not in accord with the uniform losses obtained with plant extracts and pure sugars. The uniformly higher dry weights of the excess-basic-clearing precipitates, approximately three times the weight of the excess-neutral precipitates, lends color to the co-precipitation theory.

RELATION OF QUANTITY OF PRECIPITATE TO LOSS OF REDUCING SUBSTANCES.—If the loss of reducing substances in basic clearing is due to co-precipitation alone, then the loss should be proportional to the volume of precipitate and independent of the precipitating réagent. Apple-wood extract was used for an experiment on this question because it gave the heaviest precipitate of any of the materials available. Four samples containing respectively 200, 100, 50, and 25 ml. of extract were made up to 200 ml. with 80 per cent. alcohol, the alcohol driven off and the residue taken up in water and cleared with 2 ml. of neutral-lead-acetate solution. The 100 and 50 ml. samples were best cleared by this quantity of lead and gave slightly lower results.

TABLE IV

THE EFFECT OF A NEUTRAL-LEAD PRECIPITATE ON THE RECOVERY OF REDUCING SUBSTANCES FROM APPLE-WOOD EXTRACT

QUANTITY OF EX-TRACT IN SAM- PLE, MILLI- LITERS	WEIGHT OF LEAD PRECIPITATE		REDUCING SUBSTANCES AS DEXTROSE	
	Mgs. per sample	Gms. per liter extract	Mgs. per sample	Gms. per liter extract
Two hundred	887	4.435	88.0	1.760
One hundred . . .	467	4.670	43.1	1.724
Fifty	243	4.860	21.6	1.728
Twenty-five	109	4.360	10.9	1.744

In general, however, the reducing-sugar values agree closely through a variation of 800 per cent. in weight of precipitate. In another experiment, 5 ml. of basic lead gave a precipitate weighing 890 mgs. per sample and a loss of 9.2 per cent. of the reducing value of the solution. In this case, with a larger sample, 2 ml. of neutral lead gave a precipitate of 887 mgs. in the same volume and no measurable loss of reducing substances. Apparently co-precipitation alone will not account for the observed losses.

RECOVERY OF REDUCING SUBSTANCES PRECIPITATED BY BASIC-LEAD CLEARING.—When it became apparent that loss of reducing substances in lead clearing is associated with the presence of lead oxide in the solution it seemed probable that an insoluble or difficultly soluble, lead oxide-reducing sugar compound was being formed. To determine, if possible, the fate of the lost reducing substances, five 200 ml. samples of a mangel-leaf extract were cleared with 10 ml. portions of basic-lead-acetate solution. After thorough washing with cold water the precipitates were washed from the filters with approximately 100 ml. of boiling water and refiltered. The combined hot-water extract was delead with hydrogen sulphide and reduced on a water bath to 150 cc., transferred to a volumetric flask and made to 200 ml. for a determination of reducing substances. The extracted precipitates were then combined, suspended in water and treated with hydrogen sulphide. The water-clear filtrate was made to volume and tested for reducing substances after removing the excess of hydrogen sulphide. The results are given in table V.

TABLE V

THE RECOVERY OF REDUCING SUBSTANCES PRECIPITATED BY BASIC LEAD ACETATE

SAMPLES	REDUCING SUBSTANCES IN MGS.		LOSS OR RECOVERY AS A PERCENTAGE
	Per sample	Per liter extract	
Check (low neutral) . . .	39.2	784	0.0
High basic . . .	33.9	678
Loss	<u>5.3</u>	<u>106</u>	13.5
Hot water extraction . . .	9.1	36.4	34.3
H ₂ S extraction . . .	11.7	46.8	44.2
Recovery		<u>83.2</u>	<u>78.5</u>

With the clearing given in this experiment there was a loss of 13.5 per cent. of the reducing substances or a total of 106 mgs. for the liter used. Of this loss 34 per cent. was recovered by one extraction with hot water and

an additional 44 per cent. was recovered by treating with hydrogen sulphide. The 23 mgs. not accounted for may to a large extent be assumed to have been held by the very heavy lead-sulphide precipitate which is always washed with difficulty. The completeness of the recovery and the fact that the reducing substances were apparently unchanged strongly suggested the formation of lead oxide-reducing sugar combinations which are either less soluble than the reacting substances or which are more strongly adsorbed by various precipitates. The formation of a lead compound, particularly of levulose, has been frequently mentioned in the literature. Although our data on this point are not complete enough for final conclusions, we suggest that lead oxide is the active portion of basic lead and that both ketose and aldose sugars form lead combinations, although the former perhaps more readily.

THE RELATION OF CLEARING TO THE PURITY OF THE CUPROUS- OXIDE PRECIPITATE

When reducing substances are to be estimated from the weight of cupric copper reduced, it is commonly supposed that clearing is necessary to remove, as completely as possible, precipitable organic impurities which would otherwise be retained on the filter and weighed as cuprous oxide. Investigators who have used basic lead as a clearing reagent have justified themselves on this score. We have shown that basic-lead clearing lowers the reducing value of plant extracts or pure sugar solutions from 3 to 15 or more per cent., that the loss may vary considerably from one sample to the next, and that it is roughly proportional to the degree of clearing. The losses obtained when the clearing operation was carried out on pure sugar solutions would preclude all of the difference between neutral and basic clearing being due to a lower percentage of organic impurity in the latter case, but they do not insure the efficiency of neutral clearing. The percentages of copper in the cuprous-oxide precipitates from the low-neutral or check clearing, and the high-basic or complete clearing, are assembled in table VI. Copper was deposited on a revolving grid cathode from a mixture of nitric and sulphuric acids as previously described.

All of the low-neutral solutions showed some color and most of the apple extracts were so dark as to entirely obscure the blue of the Fehling's solution. In every case the complete clearing gave a water clear solution but the tendency was for the percentage of copper in the cuprous-oxide precipitate to be lowered rather than increased by this clearing. The question of the presence of organic impurities is not closed by these data, although we were unable to detect any traces of carbon in the solutions remaining after the copper had been removed by electrolysis. If such impurities are concerned,

TABLE VI

PURITY OF CUPROUS-OXIDE PRECIPITATE FROM EXTRACTS CLEARED WITH NEUTRAL AND BASIC LEAD ACETATE

EXTRACT	PERCENTAGE OF COPPER	
	Check clearing (low neutral)	Complete clearing (high basic)
Mangel	88.18	87.81
Apple wood no. 1	87.01	86.39
Apple wood no. 2	87.61	87.30
Apple wood no. 3	87.67	87.69
Tomato	86.82	84.52
Sweet potato ..	87.42	88.04
Celery	86.03	85.60
Average	87.25	86.76
Dextrose solution ..	88.62	88.78

however, they do not belong to the group of coloring compounds which are removed by complete clearing with basic lead acetate. The percentages of copper in the dextrose precipitates were obtained under the same conditions and indicate the error due to oxidation in drying, and loss in handling. The highest value is within 0.04 per cent. of the theoretical yield of 88.82 per cent. copper, and the lower yield is probably within the error of the procedure employed.

Three points appear significant in connection with these data. (1) The percentage of copper in the cuprous-oxide precipitate of plant extracts was lower than in the precipitate of sugar solutions—both the direction and magnitude of the difference are in accord with previous observations. (2) The copper percentage value was independent of the clearing treatment and did not vary significantly between no clearing and complete clearing, although the reducing value was considerably lowered by the latter treatments. This is contrary to the reason frequently given for the need of clearing. (3) Although the percentage of copper in the plant-extract precipitates was low, the difference was small and consistent, being of the order of 2 per cent. or less. In physiological studies a constant error of 2 per cent. will not affect the conclusions to be drawn, and the gravimetric determination of reducing sugars should be satisfactory when sufficiently large quantities of cuprous oxide can be weighed to minimize fluctuations in the weights of the crucibles used. The use of basic lead acetate, on the other hand, introduces a variable error and has no advantages when reducing substances are to be determined by their action on cupric copper.

Summary and conclusions

A procedure which has given consistent results on a variety of plant extracts is recommended for the clearing of solutions to be used in the determination of reducing substances. After removing all but the last traces of alcohol from the extract the residue is taken up in warm water, allowed to cool to room temperature and sufficient neutral-lead-acetate solution, sp. gr. 1.25, added to just form a faint, white precipitate with a drop of dilute potassium-oxalate solution. Twice this quantity is used for clearing the solution tested, to insure precipitation of reducing impurities. Without standing for more than a few minutes the cleared solution is filtered onto an excess of potassium-oxalate crystals or powder. Only cold water is used for washing the lead precipitate. This method is convenient, rapid, and gives a definite end point in reducing value.

Any heating or standing in contact with a lead solution causes a rapid destruction of reducing substances. Basic lead acetate is more destructive than neutral and gives a variable loss even in cold solutions, the magnitude of which depends upon the completeness of clearing. This makes it unreliable as a clearing reagent.

The loss of reducing substances in basic clearing appears to be due to the formation of a lead oxide-sugar combination which is less soluble than the original substances and is more readily adsorbed by the clearing or deleading precipitates. The sugars may be recovered with their reducing properties unchanged by removing the lead with hydrogen sulphide.

The completeness of clearing has not affected the purity of the cuprous-oxide precipitate. In all cases where a plant extract or a mixture of extract and sugar solution was used, the percentage of copper has been approximately 87 per cent. instead of the theoretical 88.8 per cent. No good explanation for this difference is at hand. It is pointed out, however, that the magnitude and consistency of the error makes it possible to use a correction factor for gravimetric results on plant extracts or to follow the usual calculation procedures with reasonable certainty that results so obtained will be comparable, although varying perhaps 2 per cent. from the absolute values.

DEPARTMENT OF HORTICULTURE,
UNIVERSITY OF ARKANSAS,
FAYETTEVILLE, ARKANSAS

LITERATURE CITED

1. BRYAN, A. H. Report in U. S. Dept. Agr. Bureau Chem. Bull. **116**: 73. 1907.
2. EYNON, L., and LANE, J. H. The influence of alkaline earths on the determination of reducing sugars by Fehling's solution. Jour. Soc. Chem. Ind. **42**: 143T-146T. 1923.
3. LOOMIS, W. E. Some problems in the analysis of horticultural material. Proc. Am. Soc. Hort. Sci. **1924**: 365-370. 1925.
4. MEAD, G. P., and HARRIS, J. B. The gravimetric determination of reducing sugars in cane products. Jour. Ind. Eng. Chem. **8**: 504-509. 1916.
5. Methods of Analysis of the Association of Official Agricultural Chemists, p. 80. Washington, 1920.
6. QUISUMBING, F. A., and THOMAS, A. W. Conditions affecting the quantitative determination of reducing sugars by Fehling solution. Jour. Amer. Chem. Soc. **43**: 1503-1526. 1921.
7. SAWYER, H. E. Potassium oxalate as a lead precipitant in sugar analysis. Jour. Amer. Chem. Soc. **26**: 1631-1635. 1904.

THE ADAPTATION OF CERTAIN COLORIMETRIC METHODS TO THE ESTIMATION OF NITRATES, PHOSPHATES AND POTASSIUM IN PLANT SOLUTIONS¹

B. E. GILBERT

Introduction

The determination of the fertilizer requirements of individual crops has furnished a fertile field of investigation, and although many contributions have been made, much still remains to be accomplished before rational methods of fertilizer applications may be relied upon to produce normal crops. In the past the physiological chemist has attacked this problem chiefly from two angles. He has investigated the content and availability of the nutrient elements in the soil, and he has determined the total content of nitrogen, phosphorus and potassium occurring within the plant.

Both of these methods have failed to furnish a satisfactory basis for determining the mineral nutrient needs of plants. We cannot satisfactorily determine the quantity and kind of amendments needed on any given plot of land by making a soil analysis; neither can we tell by an analysis of the plants grown on the soil the amount and kind of fertilizer that should be added. This is no doubt due to the very large number of factors involved in crop production, especially with reference to the relationship between the plant and the soil in which it grows.

In this paper, work carried on during 1925-1926 is summarized, and a different mode of attack is suggested, which may prove helpful. Quite aside from the practical applications of this work, it is believed that other workers may find the methods here developed useful in studies of mineral nutrient metabolism.

Analytical methods

A. PREPARATION OF PLANT SOLUTION

✓ The procedure as given by Schreiner and Failyer (9) was adopted with certain modifications. The fresh plant tissue was shaken free from adhering soil particles, weighed and ground up thoroughly in a Nixtamel mill. The finely-ground mixture was strained through fine mesh silk and an adequate volume transferred to a small mixing bottle. With the crops investigated, a volume of 25 cc. was found to be sufficient for nitrate-nitrogen

¹ Contribution 329 of the Rhode Island Agricultural Experiment Station, Kingston, R. I.

and phosphate determinations. To this solution 1.0 gram of decolorizing carbon black for each 10 cc. of solution was added and the mixture shaken mechanically for 10 minutes. Then it was filtered by suction through an alundum extraction cone, thereby obtaining a colorless solution.✓

B. QUALITATIVE METHODS

At first it was considered possible that chemical microscopical tests on sections of the fresh tissue might be sufficiently accurate to allow the estimation of low, medium and high contents of nitrates, phosphates and potassium. Various methods were tried as given by CHAMOT (3) with indifferent success. Table I shows determinations of phosphates made with ammonium molybdate reagent; table II, the estimation of potassium with sodium cobaltinitrite, and table III, the result of the use of the diphenylamine test for nitrates.

TABLE I

MICROSCOPICAL OBSERVATIONS OF PHOSPHATE IN TISSUE OF FIELD CROPS

PLAT	RELATIVE YIELD OF MIXTURE	CROP (IN GRASS MIXTURE)	TISSUE EXAMINED	ADDITION OF PHOSPHORUS IN CHEMICAL FERTILIZER (LBS. PER ACRE)	MICROSCOPICAL OBSERVATIONS (PHOSPHATE)
65	100	Timothy	Base of stem	65	Abundant
67	62	Timothy	Base of stem	0	None
69	96	Timothy	Base of stem	22	Abundant
65	100	Red clover	Stem	65	Abundant
67	62	Red clover	Stem	0	None
69	96	Red clover	Stem	22	Abundant

TABLE II

MICROSCOPICAL OBSERVATIONS OF POTASSIUM IN TISSUE OF FIELD CROPS

PLAT	RELATIVE YIELD	CROP	TISSUE EXAMINED	ADDITION OF POTASSIUM IN CHEMICAL FERTILIZER (LBS. PER ACRE)	MICROSCOPICAL OBSERVATIONS (POTASSIUM)
41	90	Rye	Stem	43	Abundant
43	100	Rye	Stem	21	Abundant
116	3	Timothy	Stem	0	Abundant
119	100	Timothy	Stem	124	Abundant

TABLE III

MICROSCOPICAL OBSERVATIONS OF NITRATE IN TISSUE OF FIELD CROPS

PLAT	RELATIVE YIELD	CROP	TISSUE EXAMINED	ADDITION OF NITROGEN IN CHEMICAL FERTILIZER (LBS. PER ACRE)	MICROSCOPICAL OBSERVATIONS (NITRATE-NITROGEN)
17	48	Rye	Tip of leaf	0	Trace
19	70	Rye	Tip of leaf	14	Medium
21	100	Rye	Tip of leaf	28	Abundant
23 M.G. ^a	100	Spinach	Tip of leaf	135 (16 T.M. ^b)	Trace
101 M.G.	86	Spinach	Tip of leaf	84 (16 T.M.)	Trace

^a M.G.—Market garden.^b T.M.—Tons straw manure.

From the nature of the microscopical methods any determination can be considered at best but an approximation of the amount of the fertilizer element in question, and while in some cases it may be possible to estimate low and high contents, differences which may exist between these two extremes cannot be determined with any assurance of accuracy. From the data it may be noted that estimates of these extremes in the cases of nitrate and phosphate were found to be feasible. It was not, however, possible to do so with potassium. No estimations as to tissue content accompanying medium fertilizer treatment seemed possible except in the case of rye with medium nitrate fertilization. With spinach, however, such was not the case. Thus the use of such methods can only be considered as qualitative in character and not of sufficient accuracy to allow the establishing of nutrient levels accompanying the production of a normal crop.

The diphenylamine reaction for nitrates seemed to allow further elaboration and so using the decolorized plant solution, diluted portions were matched on a porcelain spotting plate against solutions of known concentration of potassium nitrate. One drop of solution was used with 8 drops of reagent (0.1 gram of diphenylamine dissolved in 10 cc. of 75 per cent. fuming sulphuric acid). The color was allowed to develop for 5 minutes before reading. Approximate estimations based on this color reaction gave correlations between color developed and fertilizer treatment as shown in table IV. The diphenylamine reaction has been shown by JAEGER (8) and others to be inaccurate as a measure of nitrates due to the dependence of the color production upon an oxidation reaction. Thus the presence of other oxidation reagents than nitrate introduces an appreciable error. Realizing this difficulty the diphenylamine reaction was discarded.

TABLE IV

COMPARISON OF NITRATE-NITROGEN CONTENT OF PLANT SOLUTION AND
NITROGEN ADDED AS CHEMICAL FERTILIZER

PLAT	RELATIVE YIELD	CROP	TISSUE EXAMINED	ADDITION OF NITROGEN IN CHEMICAL FERTILIZER (LBS. PER ACRE)	NITRATE- NITROGEN IN PLANT SOLUTION (PPM)
113 M.G. ^a	100	Cabbage	Midribs	75 (16 T.M. ^b)	2085
112 M.G.	92	Cabbage	Midribs	50 (16 T.M.)	1737
116 M.G.	100	Tomatoes	Leaves	75 (16 T.M.)	973
115 M.G.	91	Tomatoes	Leaves	50 (16 T.M.)	814
Sec. 1 C.A. ^c	85	Corn	Stem internodes	20	Trace
Sec. 3 C.A.	100	Corn	Stem internodes	60	695

^a M.G.—Market garden.

^b T.M.—Tons straw manure.

^c C.A.—Corn acre.

C. QUANTITATIVE METHODS

✓ 1. *Nitrate-nitrogen*.—The phenol di-sulphonic acid method for the estimation of nitrates as outlined by HARPER (6) for soils was found to be applicable when modified slightly (table V). To a volume (dependent upon the nitrate content) of the clarified solution 10 cc. of a saturated solution of silver sulphate, 0.5 cc. of normal cupric sulphate, 0.2 gram of calcium hydrate, and 0.5 gram of magnesium carbonate were added. The mixture was shaken at intervals for 5 minutes, heated to boiling, cooled, made up to volume and filtered. Of the filtrate a 10 cc.-aliquot was evaporated on the water bath to dryness, the phenol di-sulphonic acid reagent added and the remainder of the procedure carried out as outlined by HARPER. The final color was compared in a Kennicott-Sargent colorimeter against the color developed by a solution of potassium nitrate containing 1 part per million of nitrate-nitrogen.

The addition of silver sulphate is necessary due to the presence of appreciable amounts of chloride in the plant solution; copper sulphate serves to precipitate soluble organic matter; the colloidal silver chloride precipitate is coagulated by means of the calcium hydrate and magnesium carbonate, and any excess of silver ion is also thrown out of solution. This method has been found to be capable of swiftness of manipulation, ease of duplication, and an accuracy sufficient to show small differences in nitrate content.

✓ 2. *Phosphate*—The coeruleo-molybdate method of DENIGÉS (4) as modified by FLORENTIN (5) and used for the determination of phosphate content of soil extracts by ATKINS (2) was found to afford a rapid and accurate

method for the estimation of the phosphate content in plant solutions (table VI). The color was developed in diluted aliquots of the clarified solution and the comparisons made by means of a Spencer-Duboseq colorimeter against a standard solution of potassium di-hydrogen phosphate having a phosphorus content of 0.25 part per million.

Since appreciable amounts of silicon occur in the plant solutions and since with certain concentrations of silicon a color similar to that obtained with phosphate is developed, it seemed advisable to ascertain to what extent silicon might interfere. Gravimetric silicon determinations were made (1) on a number of plant solutions and the silicon content found to vary from 130 to 350 parts per million of solution. The presence of silicon in solutions prepared *in vitro* from sodium silicate and containing a determined concentration of silicon, above 25 parts per million, was found to give a color which could be observed in the colorimeter. However, since dilutions ranging from 100 to 400 were found necessary in many cases to render the concentration of phosphate of sufficient dilution to allow a comparison in the colorimeter, the final concentrations of silicon in the solutions measured were always much below those liable to develop a color with the reagent. In order to further investigate the possibility of change of tint due to presence of silicon, the color was developed in solutions containing 0.25 part per million of phosphorus plus 21 parts per million of silicon, 0.25 part per million of phosphorus plus 32 parts per million of silicon, and 0.25 part per million of phosphorus, the latter having been used as a standard for comparison. The solution containing 21 parts per million of silicon gave the same depth of color as the standard while the solution with 32 parts per million of silicon was slightly deeper in color. FLORENTIN (5) has noted a similar differential sensitivity with phosphorus and silicon in determinations made on natural waters.

3. *Potassium*.—The method devised by HILL (7) and advocated by SCHREINER and FAIRYER (9) based on the estimation of the yellow color produced by the reduction of potassium chloroplatinate with stannous chloride in the presence of free hydrochloric acid, was used (table VII). Determinations were made using 5 cc.-portions of the plant solution before clarification.

This method was found to be easily adaptable for use with the Kennicott-Sargent colorimeter and has both simplicity and accuracy in its favor. However, certain precautions were found necessary. The potassium content of the solution under investigation must govern the volume used, as too great a concentration of chloroplatinate will interfere with the final color produced on reduction. Extreme dilution of the chloroplatinate solution before reduction causes a turbidity to develop which interferes with the final color determination.

It should be noted that extreme accuracy is not claimed for these methods due to all the errors accompanying colorimetric methods of which the occurrence of "off-tints" is perhaps the most difficult to control. But with careful manipulation and the observance of certain precautions such as the development of the standard and sample colors at the same time, these inherent errors may be minimized. On the other hand, however, it is claimed that the use of these methods enables the worker to estimate small concentrations, establish small differences, and give him a means of securing ease and rapidity of manipulation so that many samples may be determined with a minimum of time and labor.

Analytical determinations

Crop plants growing under similar conditions as to climate, soil P_H , and period of growth were chosen for analysis. In so far as was possible crops were chosen in which the only variable factor during growth was the amount of one fertilizer ingredient. Each sample consisted of as many individuals as was consistent with the material obtainable, and ease of manipulation. During the months of November to February inclusive, plants were also grown in the greenhouse. Soil was transferred from three field plats to the greenhouse. The soil from one plat was known to be low in active P_2O_5 ; that from the second low in nitrate-nitrogen; and the third low in K_2O . Determinations for active P_2O_5 and nitrate-nitrogen were made on the first two soils. The soil low in phosphate yielded 44 parts per million of active P_2O_5 after a 1-hour extraction with 2N acetic acid. The low nitrate soil was found to contain 11 parts per million of nitrate-nitrogen. To each of

TABLE V
COMPARISON OF NITRATE-NITROGEN CONTENT OF PLANT SOLUTION AND
NITRATE-NITROGEN ADDED AS CHEMICAL FERTILIZER

PLAT	CROP	TISSUE EXAMINED	ADDITION OF NITROGEN IN CHEMICAL FERTILIZER (LBS. PER ACRE)	NITRATE-NITROGEN CONTENT IN PLANT SOLUTION (PPM)
Greenhouse	Lettuce	Leafy	0	Trace
Greenhouse	Lettuce	Leafy	60	225
Greenhouse	Lettuce	Leafy	120	523
Greenhouse	Tomatoes	Leaves and petioles	70	560
Greenhouse	Tomatoes	Leaves and petioles	53	350
Greenhouse	Rye	Leafy	0	33
Greenhouse	Rye	Leafy	60	50
Greenhouse	Rye	Leafy	120	80

these soils three levels of each fertilizer element were added and crop plants grown. The quantitative methods as outlined above were employed and a few of the results obtained are given in tables V to VII.

TABLE VI

COMPARISON OF PHOSPHORUS CONTENT OF PLANT SOLUTIONS AND PHOSPHORUS ADDED AS CHEMICAL FERTILIZER

PLAT	RELATIVE YIELD	CROP	TISSUE EXAMINED	ADDITION OF PHOSPHORUS IN CHEMICAL FERTILIZER (LBS. PER ACRE)	PHOSPHATE-PHOSPHORUS CONTENT IN PLANT SOLUTION (PPM)
108	82	Corn	Stem tips	0 (2.3 T. M. ^a)	2
107	90	Corn	Stem tips	26 (2.3 T. M.)	34
112	100	Corn	Stem tips	52 (2.3 T. M.)	78
79	90	Corn	Stem tips	39 (1924)	43
77	100	Corn	Stem tips	59 (1924)	60
Greenhouse		Turnips	Leafy	0	32
Greenhouse		Turnips	Leafy	60	42
Greenhouse		Turnips	Leafy	120	93

^a T. M.—Tons shavings manure.

TABLE VII

COMPARISON OF POTASSIUM CONTENT OF PLANT SOLUTION AND POTASSIUM ADDED AS CHEMICAL FERTILIZER

PLAT	RELATIVE YIELD	CROP	TISSUE EXAMINED	ADDITION OF POTASSIUM IN CHEMICAL FERTILIZER (LBS. PER ACRE)	POTASSIUM CONTENT IN PLANT SOLUTION (PPM)
119 M. G. ^a	100	Celery	Stems	83 (16 T. M. ^b)	900
89 M. G.	89	Celery	Stems	124 (16 T. M.)	1100
113 M. G.	84	Beets	Roots	83 (16 T. M.)	612
83 M. G.	100	Beets	Roots	124 (16 T. M.)	812
115 M. G.	93	Spinach	Leaves	83 (16 T. M.)	1350
86 M. G.	100	Spinach	Leaves	124 (16 T. M.)	1450
Greenhouse		Lettuce	Leaves	0	1641
Greenhouse		Lettuce	Leaves	100	2116
Greenhouse		Lettuce	Leaves	199	2777

^a M.G.—Market garden.

^b T. M.—Tons straw manure.

Discussion

The individual colorimetric methods have already been considered. There remains the discussion of the practical application of these methods. It is recognized that any method of determining the fertilizer needs of plants is beset with difficulty. The data at present are insufficient to give us any idea of the amount of variability to be expected in the concentration of mineral nutrients in the sap of normal crop plants. With reference to mineral nutrient cultures, it has been shown that there is no single best mineral nutrient ratio for the growth of any kind of plant in such culture solutions. Over rather wide ranges of variations of these ratios the growth of plants is about equally good. Similarly we do not know at present how wide may be the variation of the ratios of N:P:K in the cell sap, and still have normal crop production. However, it was felt that determination of the soluble nutrients in the cell sap might furnish a more reliable clue to the nutrient needs of plants for normal growth than the older methods did. There are some indications in the data presented that the methods here employed may have value in this connection. Thus in tables VI and VII there is some correlation shown between yield and the amount of nutrient element found in solution in the cell sap. Hitherto attempts have been made to secure information regarding nutrient levels mainly by making total analytical determinations on plant tissues. One would expect that a determination of the amount of nutrient elements actually present in solution, and capable of entering into metabolic combinations, should be a better method of delimiting such nutrient levels. This should be true, particularly if determinations are made at intervals throughout the growth of the plant, and coupled with growth measurements and total yield. If by such methods we can determine what constitutes a proper nutrient level for normal crop production, and how to change the nutrient level by soil amendments, these same methods may be of use in maintaining these levels by providing information as to the amount of fertilizer needed, and the proper time for application during the development of the plant.

Summary

In this paper are discussed certain colorimetric methods for the determination of nitrate-nitrogen, phosphate-phosphorus and potassium in the plant solution and the practical applications arising from their use.

1. *Qualitative.* The usual chemical microscopical tests were found to be incapable of use in the establishing of low, medium and high nutrient levels in crop plants.

2. *Quantitative.* The following methods were found to be applicable to determinations with plant solutions:

- (a) Nitrate-nitrogen.—Phenol di-sulphonic acid method.
- (b) Phosphate-phosphorus.—Coeruleo-molybdate method.
- (c) Potassium.—Reduced chloroplatinate method.

3. The application of these methods to the establishing of optimum nutrient levels of the three fertilizer elements within the plant as indicative of a normal crop is discussed.

The writer is indebted to Dr. B. L. HARTWELL for his kindly criticism and encouragement during the progress of this work.

RHODE ISLAND AGRICULTURAL EXPERIMENT STATION,
KINGSTON, R. I.

LITERATURE CITED

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. *Methods of Analysis*. 1919.
2. ATKINS, W. R. G. The rapid determination of available phosphate in soil by the coeruleo-molybdate reaction of Denigés. *Jour. Agr. Sci.* **14**: 192–197. 1924.
3. CHAMOT, E. M. *Elementary chemical microscopy*. John Wiley & Sons. 2nd ed. 1921.
4. DENIGÉS, G. Détermination quantitative des plus faibles quantités de phosphate dans les produits biologiques par la méthode céruleo-molybdique. *Compt. Rend. Soc. Biol. (Paris)* **84**: 875–877. 1920.
5. FLORENTIN, D. Détermination de phosphate dans l'eau. *Ann. Chim. Anal. Chim. Appl.* **3**: 295–296. 1921.
6. HARPER, H. J. The accurate determination of nitrates in soils. *Jour. Ind. Eng. Chem.* **16**: 180–183. 1924.
7. HILL, L. A. A colorimetric method for the determination of small quantities of potassium. *Jour. Amer. Chem. Soc.* **25**: 990–992. 1903.
8. JAEGER, G. Die Eisen-(II)-sulfat-Probe auf Nitrat und Nitrit und die Diphenylamin-Reaktion. *Ber. D. Chem. Ges.* **58B**: 2340–2343. 1925.
9. SCHREINER, O., and FAILYER, G. H. Colorimetric, turbidity and titration methods used in soil investigations. *U. S. Dept. Agr. Bur. Soils Bull.* **31**. 1906.

BRIEF PAPERS

SOME FURTHER NOTES ON THE HYDROLYSIS OF STARCH GRAINS UNDER POLARIZED LIGHT

(WITH ONE PLATE)

The interest taken by workers in America in the effects of radiation of all kinds and in particular of polarized light, together with the confirmation of my results, kindly expressed by Prof. MACHT and Mr. MORRISON, lead me to hope that some fuller account of these experiments may be acceptable.

The first paper published in Dec., 1924,¹ was necessarily of a preliminary character, but recent work has completely confirmed these early results and has made the whole process much clearer.

Like all new ideas, this work has not escaped criticism,² to which a reply³ has been given, and it is hoped that the following added notes and photographs will show that the views put forward by our critics are quite untenable.

1. As stated in the above mentioned paper, washed and centrifuged starch grains were suspended in water, to which weak diastase was added and a few drops of the starch suspension were mounted on a microscope slide placed over a Nicol prism. Two controls were always arranged, one in ordinary light, and the other in darkness. It has been suggested that the whole effect was due to pressure of the cover slip, but this factor would have affected the controls also.

2. In the second place, it is most important to note that the whole process was watched carefully under the microscope for four or five hours, the changes were noted and *drawings* were made. Unless this is done, a superficial observer may easily miss the results, particularly if no cover slip is used, as the grains on hydrolysis become lighter, exude their contents, and the empty coats float to the edge of the slide or vessel.

3. The experiment was repeated nearly twenty times, and the result was shown to nine or ten different observers, chiefly professors and lecturers of the University of London.

¹ BALY, E. C. C., and SEMMENS, ELIZABETH S. The selective photochemical action of polarized light.—I. The hydrolysis of starch. *Proc. Roy. Soc. London B.* **97**: 250–253. 1924.

² JONES, NEILSON. Polarized light and starch grains. *Ann. Botany* **39**: 651–653. 1925.

³ BALY, E. C. C., and SEMMENS, ELIZABETH S. Selective action of polarized light upon starch grains. *Nature* **116**: 817. 1925.

4. The first change was seen as a light transparent streak, evidently *within* the grain and starting from the hilum. This increased, and branches were formed extending to the exterior. These are seen on plate VIII, figs. 5 and 6.

5. The whole grain became lighter and more transparent and the edges began to split. Figs. 7 and 8. (This is also seen in fig. 3 where the effect was produced by polarized light alone, acting on well-washed grains in distilled water.)

6. The grain started to swing very slowly from side to side and in some cases moved its position, owing probably to exudation of the hydrolyzed contents of the grain and to changing surface tension. This fact shows that the change observed could not have been due to pressure of the cover slip.

7. Under crossed Nicols the huminescence of the grain gradually disappeared from the center towards the periphery. Fig. 9.

8. The familiar black cross became blurred in the same way and finally disappeared.

9. On drying, a cluster of crystals formed *at the point where the grain had been* and only the empty shrivelled or broken coat or shell remained. This sometimes became so transparent as to be nearly invisible. Figs. 10 to 12. The arrangement of these crystals most probably depends on the shape of the original starch grain.

10. On addition of Fehling's solution, as before stated, the presence of a reducing substance was indicated. The photomicrographs, figs. 1 and 2, are submitted as showing the results better than those in the original paper. They were not published at the time, as one of the controls was spoiled.

In both controls, however, the whole field on the slide was searched and only two grains were found imperfect on each slide. In fig. 1, over the polarized light, the grains were commencing to break all over the field exposed.

Fig. 3 illustrates the change within the grain before any serration has taken place, except that little canals are forming to the exterior. This result was obtained with long weak exposure to polarized light. No diastase was added to the starch suspension. In fig. 13 this change has gone further; the contents of the grain are forming crystals and the shell is beginning to crack. In fig. 4 and fig. 14 the coat has broken down and the hydrolyzed contents have exuded into the surrounding liquid.

In conclusion, it may be noted that if, as Professor JONES suggests, all these results are due to accidental pressure of the coverslip (even when the exposure took place in a flask!) then there is a by fortune to be made in the sugar industry. The only requisites are a few tons of corn or potatoes and a steam-roller!—ELIZABETH SIDNEY SEMMENS, *Bedford College, University of London.*

EXPLANATION OF FIGURES.

FIG. 1. Potato starch exposed to light polarized by a Nicol prism. All stages of hydrolysis are present. (See figs. 5-12.)

FIG. 2. Control of potato starch exposed to ordinary light. Grains A and A' appear to be fractures, as there is no transparency in the middle, which is the mark of hydrolysis.

FIG. 3. Starch grains exposed in flask to polarized light through base of flask, showing hydrolysis of contents, probably to dextrin. C, C: canals to exterior.

FIG. 4. Starch grains exposed to polarized light, with diastase, showing exudation from grain. The coat of the grain is left in the center.

FIGS. 5-12. Typical stages of hydrolysis of starch grains exposed to polarized light, with diastase. Note transparent streak in fig. 6, the rod-like crystals from the shell of the grain in fig. 10, and the radiating crystals surrounding the shell in figs. 11 and 12.

FIG. 13. Washed starch grain in distilled water, after several days' exposure, in a flask, to polarized light. Note crystals formed inside; also slits in the coat.

FIG. 14. Starch grains, prepared and exposed as in fig. 1. Note exudation from the grain crystallizing out.



Fig. 1

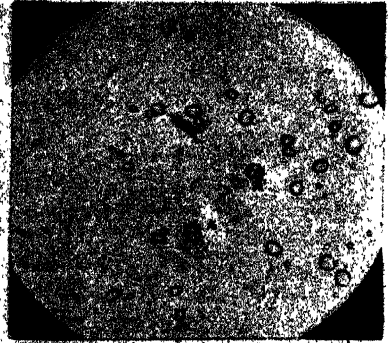


Fig. 2

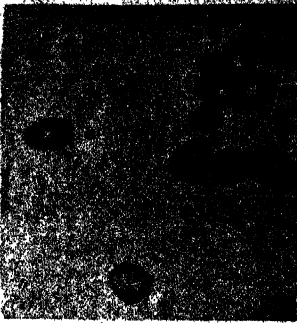


Fig. 3

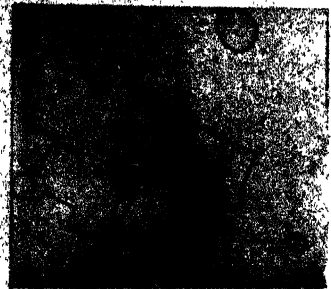


Fig. 4



5



6



7

Figures 5 to 12



8



9



10



11



12



Fig. 13

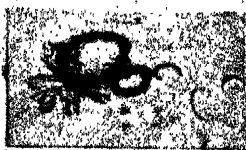


Fig. 14

A CONDUCTIVITY CELL FOR CONTINUOUS MEASUREMENTS OF RESPIRATORY RATE

(WITH ONE FIGURE)

The conductivity method is very desirable for continuous readings of the rate of CO_2 production when rapid fluctuations in the rate of respiration are under investigation. There have been devised by CAIN and MAXWELL¹ and by SPOEHR and MCGEE² conductivity cells which make use of the change in conductivity of $\text{Ba}(\text{OH})_2$ solutions for the estimation of carbon dioxide. The apparatus here described (fig. 1) has some advantages

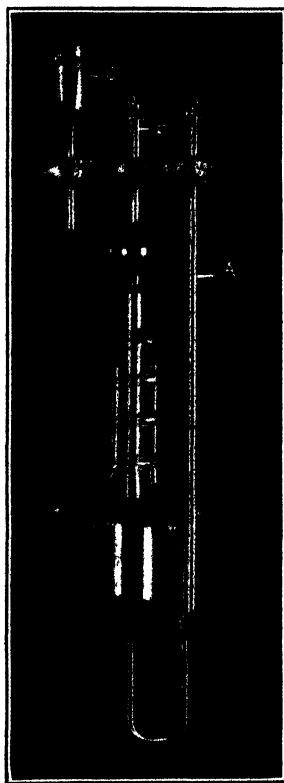


FIG. 1. Conductivity cell for respiration studies. See text for description.

¹ CAIN, J. R., and MAXWELL, L. C. An electrolytic resistance method for determining carbon in steel. *Jour. Ind. Eng. Chem.* 11: 852-860. 1919.

² SPOEHR, H. A., and MCGEE, J. M. Studies in plant respiration and photosynthesis. *Carnegie Inst. Washington. Publ. no. 325.* 1923.

over the former types since it can be made small and compact and is not easily breakable. The apparatus can be shunted into a closed system of air circulation or the CO_2 may be drawn through it by vacuum.

The gas enters by the long tube (A) at the right, rises through the spiral in interrupted bubbles mixed with the $\text{Ba}(\text{OH})_2$ solution, and passes out the center tube (B) which is provided with a bulb to break up the films. The absorbing solution is thoroughly mixed and continually circulated through the spiral. The funnel side tube (C) is useful for filling the apparatus. Connections to the electrodes are made through glass tubes at the sides. The cell is made of pyrex glass and the change of the capacity factor is found to be negligible since each electrode is protected from the deposition of BaCO_3 on it by a projecting glass collar. The electrode vessel is filled with a known volume of $\text{Ba}(\text{OH})_2$ solution (100 cc.) so that the level stands below the top of the spiral when the cell is in operation. The concentration of the $\text{Ba}(\text{OH})_2$ solution may be varied within the limits of the capacity of the electrodes. Usually N/20 to N/200 $\text{Ba}(\text{OH})_2$ is used. The cell is arranged for immersion in a water thermostat.

We have found this cell very useful for measuring quick changes in the rate of respiration of tissues resulting from treatment with ethylene and other gases.—R. B. HARVEY AND L. O. REGEIMBAL, *University of Minnesota*.

CITATION OF LITERATURE

As science progresses there is a constantly increasing number of investigators who need the services of scientific periodicals in placing the results of their research before the public. This is true of botany and plant physiology, just as it is of all the other branches of science. The volume of literature produced by this constantly augmenting army of workers has grown correspondingly. One is reminded, in this connection, of those autocatalytic processes in which a product of the reaction catalyzes, and increases the rate of the reaction. We have the machinery for investigation in operation, and one of the products of this system is the trained investigator. He is the catalyst, who is constantly accelerating the rate and volume of scientific output.

With this increase in the number of workers and volume of literature, the editors and publishers of journals find themselves confronted by various serious problems. One of these is the citation of scientific literature. In the early years it was necessary to bring together far scattered materials, and focus attention upon the bearings of these fragmentary contributions. So the custom grew up of citing the literature rather completely. It was a useful and necessary part of scientific publication. Then the literature was scanty in all fields; today there are only a few fields in which the published

literature is scanty, especially in connection with the larger problems of biology. It must be perfectly obvious that we shall not be able to continue indefinitely the custom of giving complete citations to the literature of any given field. We shall soon reach the place where the literature citations will occupy more space than our contributions warrant. This situation should be given careful and deliberate consideration by investigators, editors and publishers.

Investigators can render assistance by using more discrimination in the citation of papers. There is no particular virtue in the long list of citations as such. Occasionally one sees the results of over-zealous listing of literature which hardly deserves mention, and the inclusion of papers that have but little bearing on the problem under discussion. The writer has in mind a case which came to his attention some time ago. It is mentioned only to provide a concrete example of a method to be avoided. Buscalioni and Pollacci in 1904 published a lengthy paper on "The anthocyanins and their biological significance in plants." The references to the literature numbered well toward 900 even at this early date, and occupied about 30 pages, just for citations alone. Among these citations was noted one on "Blue Ridge Blossoms." While some of the plants mentioned in this species list had anthocyanin-containing flowers, the paper was probably included because it had the word *blue* in the title. The paper cited had nothing to do with the biology of the anthocyanins. A discriminating consideration of the value of papers would have led to the omission of those which had no bearing on the problem.

It is necessary, of course, to *know* the literature as completely as possible. Nothing can quite take the place of a complete bibliography in the hands of the individual workers. But when we come to report our results we can conserve space by limiting ourselves strictly to the citation of only such papers as cannot be omitted from the discussion without loss in value.

It is frequently possible to reduce the number of citations by reference to other recent papers where most of the earlier papers have been summarized. By such measures, and judicious choice of citations, one may greatly reduce the number of papers that must be mentioned, and so reduce the cost of publication.

The editors and publishers of this journal invite the cooperation of those who use its pages as a medium of communication with other botanists in a program of true economy, which does not sacrifice quality, but which eliminates as far as possible any unnecessary waste of labor and space.—C. A. SHULL, *Editor-in-Chief*.

NOTES

The International Congress of Plant Sciences.—It is now possible to mention the names of some of the distinguished foreign scientists who will attend the Ithaca Congress, at Cornell University, Aug. 16–23, 1926. Plant physiologists will note with pleasure the inclusion of such names as Stoklasa, Lepeschkin, Maximow, Chodat, Němec, Winogradsky, Prianishnikow, Lubimenko, and many others. There are still others who would come if they could secure financial aid through giving lectures before American scientific audiences. It would be a very appropriate and gracious form of hospitality to extend invitations to some of these noted men, who are hesitating to come because of financial reasons, to lecture before the students and faculties of our respective institutions. Can we not use this opportunity to foster a warmer international cordiality among scientists? Science knows no national boundaries nor racial prejudices. It serves humanity impartially. We want to give our distinguished guests a real heart-warming experience during this visit to America.

Those who would like to extend invitations to visitors who would thus be enabled to attend the Congress can secure information as to where such assistance would be welcome from Dr. B. M. Duggar, Chairman of the Executive Committee, and General Secretary of the Congress, who should be addressed, National Research Council, B and 21st Sts., N.W., Washington, D. C.

Membership in the A. A. A. S.—The Council of the American Association for the Advancement of Science voted at the Kansas City meeting to remit the entrance fees to all members of affiliated societies during the next three years. This is an opportunity which will no doubt appeal to the new members of all of the affiliated societies. Election to membership in one of the affiliated societies should be considered an invitation to membership in the general Association.

Life Membership Fund.—"The American Society of Plant Physiologists . . . has established a life membership fund of \$2,000, in honor of Dr. CHARLES REID BARNES, who was, at the time of his death in 1910, Professor of Plant Physiology at the University of Chicago. The fund has been established in accordance with plans accepted by the Society at its second annual meeting at Kansas City. The interest of this fund will be used each year to elect to life membership in the Society some member whose contributions to Plant Physiology make him most worthy to receive the honor.

The members so elected will be known as the Charles Reid Barnes Life Members. The first election will be held in connection with the third annual meeting at Philadelphia in December, 1926."—*Science*: Feb. 26, 1926.

Summer Meeting.—The Corn Belt Section of the American Society of Agronomists will meet at the University of Minnesota, July 15–17, 1926. Last year the American Society of Plant Physiologists met with the Agronomists at Michigan State College, East Lansing, and the joint meeting was greatly enjoyed by those in attendance. An invitation has been extended to the Physiologists to meet again with the Agronomists. A little later the Horticulturists are planning a meeting at the University of Minnesota. It would have been a splendid arrangement if these several groups could have met together, since the fields are so closely allied, but such a joint meeting of all three probably cannot be held this year.

In view of the fact that the International Congress of Plant Sciences meets at Ithaca in August, it is hoped that no one will sacrifice the meeting at Ithaca for other meetings. However, these regional meetings of allied groups for good fellowship and mutual stimulation are worth while, and it would be fitting for the Physiologists to hold a joint regional meeting with the Agronomists at Minneapolis. Announcements will no doubt be sent out to the members if such a regional meeting is arranged.

The Purdue University Section.—To Purdue University goes the honor of organizing the first Local Section of the American Society of Plant Physiologists. The organization of such a section requires the presence of ten or more members in the local group, and Purdue is to be congratulated on having so large a group interested in the promotion of research in plant physiology. The Purdue Section has been meeting weekly, the meetings being delightfully informal, and the group is enthusiastic over the results of the early meetings. Dr. E. B. MAINS is the local Vice President, and becomes *ex-officio* a member of the Executive Committee of the Society. No other officers have been elected. It is a great pleasure to welcome this first Local Section, and we hope that many such sections may be organized among the investigators in our larger universities.

The University of Minnesota Section.—An application for a Local Section at the University of Minnesota has been received. This application will be acted upon in due time. It is very encouraging to see the plant physiologists uniting to encourage one another in the investigation of important physiological problems, and to enjoy the fellowship of common interests. There are many ways in which such a group of investigators can cooperate to the advantage of each individual in it, and of the program of research as a whole. It should be definitely easier to maintain the spirit of

enthusiastic research where men are banded together in this way for mutual helpfulness.

Research Libraries.—Every investigator finds it necessary to own a library made up of books which are indispensable aids to research. Among these there are always some which must be imported. Those who need the services of an importer will find G. Schmidt, 415 Woodland Ave., Leonia, N. J., and B. Westermann Co., 13 West 46th St., New York, courteous and prompt in caring for import orders. Those who desire to send directly to Europe will find Oswald Weigel, Königsstrasse 1, Leipzig, Germany, reliable and accommodating to purchasers of books.

Chemical Bibliographies.—The National Research Council, Washington, D. C., has published a bibliography of bibliographies on chemistry and chemical technology. The work has been compiled by WEST and BEROLZEIMER, and is published as Bulletin no. 50 (308 pp., \$2.50). There are a number of sections, including general bibliographies, abstract journals and year books, general indexes of serials, bibliographies of special subjects, and personal bibliographies.

The research chemists and chemical physiologists will find this work of assistance in locating bibliographies relating to any subject in which they are interested. The literature lists from research papers, and even footnote bibliographies have been compiled, giving the name of the author, title of paper, place of publication, and frequently the number of citations listed.

The references are classified according to proper subject headings alphabetically arranged. There are about 2,400 subject headings, 7,500 author entries, and approximately 10,000 individual bibliographies. The volume should find a place in the library of every one who may need to consult the literature of chemistry. Omissions no doubt occur, but the book offers an excellent starting point for the necessary delving into the literature in connection with research.

Chemical Plant Physiology.—The rapid advances made in plant physiology during the last 25 years constitute a revolution almost as extensive as that which occurred in chemistry during the last quarter of the eighteenth century, and as striking as the revolution which has taken place in physics during the last three decades. In the first volume of his new *Lehrbuch der Pflanzenphysiologie*, KOSTYTSCHEW points out the fact that a comparison of his book with the same chapters in PFEFFER shows that little is left of the physiology of 20 years ago, "ein kaum zu erkennendes Gerüst," and that innumerable new problems have come up for solution. These enormous changes have not yet been recorded sufficiently in text books, especially in

English. KOSTYTSCHEW has attempted, in this volume on chemical physiology, to present the new science in appropriate form. The eight chapters in volume I are logically arranged, and deal with the following topics: The foundations of chemical plant physiology; the assimilation of sunlight energy by green plants, and the primary synthesis of organic matter; chemosynthesis and the assimilation of molecular nitrogen; the nutrition of plants by organic compounds; the nutrition of plants by ash elements and its significance; carbohydrates and proteins, their transformations in the plant; the secondary plant constituents; and respiration and fermentation.

The book is thoroughly modern in its point of view, and should be examined by every physiologist. KOSTYTSCHEW still clings to the ten necessary elements, a point of view that is becoming untenable as we more carefully exclude elements needed in minute quantities. McHARGUE's work on manganese is not mentioned in the discussion of this element, and evidence is accumulating that other elements are necessary in minute amounts, possibly as small as a part in several millions of water. Perhaps we should no longer try to list a definite number of required elements.

Official and Tentative Methods of Analysis.—The 1925 edition of this well known publication of the Association of Official Agricultural Chemists is now available for distribution. The revision has been thorough, and includes the additions, deletions, and other changes made at the meetings of the Association from 1919 to 1923. The book contains nearly 100 pages more material than the 1920 edition, and two additional chapters, on agricultural liming materials, and gelatin. The arrangement of chapters has been changed at several places, soils now following immediately after fertilizers, after which comes the new chapter on liming materials. This is a better arrangement. The price is \$5.00, and may be ordered from W. W. SKINNER, Secretary, Box 290, Pennsylvania Avenue Station, Washington, D. C.

Chemical Action of Ultraviolet Rays.—A useful summary of the enormous literature on ultraviolet radiation has been compiled by ELLIS and WELLS. Plant physiologists will find many of the chapters illuminating, although they deal with other than strictly biological effects. The book contains fifteen chapters, the most valuable of which are those dealing with photochemical mechanism, photochemical and photolytic reactions, photosynthesis, sterilization, and the biologic effects. A few errors will be found by the critical reader, and one has the impression that the authors have taken too seriously some of the highly theoretical and hypothetical papers on the relation of ultraviolet radiations to photochemical reactions occurring

in plants. Any student of the ultraviolet as a factor in biological processes will find it an indispensable aid, in spite of any shortcomings it possesses. It is published by the Chemical Catalog Co., New York.

Physiological Basis of Drought-Resistance of Plants.—This volume by Prof. N. A. MAXIMOW, published in Russian by the Institute of Applied Botany and New Cultures, Leningrad, 1926, represents a critical survey of the literature on water balance and drought resistance, supplemented by investigations of the author and his coworkers in this field. The first part deals with the processes of absorption of water in the soil; the second, with transpiration phenomena. The third part discusses water balance, water deficit, and the problems of drought resistance. The book is valuable in emphasizing the anatomo-morphological characters as well as the physiological characters of drought-resistant crops. While the main text will remain a sealed book to most American students, it has a brief abstract in English, fifteen pages, at the close. Negotiations are under way for an English translation, slightly abridged, under the editorship of Prof. Yapp, of the University of Birmingham. The Russian edition may be obtained by addressing the Institute, Rue Herzen 44, Leningrad, U. S. S. R. The price is \$2.50.

PLANT PHYSIOLOGY

JULY, 1926

THE MECHANISM OF ACCUMULATION OF DYES BY LIVING CELLS

G. W. SCARTH

Introduction

While this paper deals with the storage rather than the uptake of dyes, it may be stated at the outset that the writer's experiments support the view that rate of entry is often largely determined by and increases with the capacity for storage, while on the contrary the rate at which dyes leach out of the cell decreases with this capacity. The present object is to explain the mechanism of accumulation and retention of dye once it has entered, rather than that by which it enters or leaves the cell. There is no doubt that BETHE (1) and NIRENSTEIN (19) went too far in ascribing all differences in penetration of dyes to their differential affinity for cell colloids. RUHLAND had mentioned this factor but finally (23, 24, 25) laid stress on diffusibility (size of diffusing particle). In this he also took too limited a view, for while, with his experimental method, viz.: of allowing dyestuffs to ascend with the transpiration current and subsequently filter through the tissue, the rate at which dyes appear in the cells follows approximately the order of their rate of diffusion through filter paper, yet, when dyes are applied directly to cells, and in concentrations not too high, the superior penetration of basic over acid dyes is a matter of common experience. From dilute solution the latter often enter so slowly that it requires special methods such as COLLANDER (4) employed to detect their presence in the cell even after two or three days exposure. Within each group of dyes, however, rate of penetration does appear to depend largely on diffusibility.

There are three classes of theory as to the storage of dyes, (a) physical, (b) chemical and (c) physiological.

(a) The physical class includes principally the adsorption theory such as BETHE argued for basic dyes and RUHLAND for acid. RUHLAND while making no distinction as to the mode of entry of the two classes of dye does

make a distinction as to storage (*speicherung*), saying that acid dyes while penetrating quickly take a long time to pass into a state in which they are held by the cell, but, when they do, are difficult to wash out; whereas basic dyes pass more speedily into this state but are easier to wash out. With the first part of this statement the writer's results agree, but in all the cases studied, basic dyes, when they accumulate in much higher concentration in the sap than outside, are held little less strongly than the acid variety. In fact it has only been with the onset of moribund changes in the cell that the accumulated dye, whether basic or acid, has appreciably diminished in concentration. This has been noted for example in epidermal cells and stomata of *Allium*, *Tradescantia*, etc., and in sections of tissue of various plants.

There is therefore no reason for supposing on the ground of tenacity of retention that the two classes of dyes are held by an essentially different mechanism, although it is significant that the ability to accumulate the respective types frequently belongs to different cells. In the colored epidermis of *Tradescantia*, for example, it is the unpigmented cells, *e.g.*, stomatal guard cells, that rapidly accumulate neutral red, etc., the colored cells taking it up more gradually with mutual precipitation of the added (basic) and the natural (acid) pigments. A more specific type of colloidal combination involving chemical as well as physical union is postulated in NIRENSTEIN'S lipid absorption theory.

Opposed to the mechanism of combination with a colloid we find COLLANDER, who argues, for example, that the cell sap is generally poor in colloids. Also OSTERHOUT'S (20) and HOAGLAND and DAVIS'S (5) analyses of the cell sap of *Valonia* and *Nitella* indicate that ions accumulate there in a freely diffusible state, which seems to deprive the "colloidal combination" theories of any element of inevitability.

(b) As regards chemical theory, PFEFFER presented evidence for combination of basic dye with acids produced by the cell, and RUHLAND added the idea that the action was reversible, to account for the relation of equilibrium between the external and internal concentrations. More recently OSTERHOUT (21, 22) and IRWIN (10, 11, 12, 13) have argued on the basis of quantitative tests, for an even simpler chemical change, *viz.*: the dissociation of the dye hydrate—which alone is supposed to penetrate—dissociation being normally greater internally than externally owing to the lower P_H of the sap.

The great objection to the above and other (such as DONNAN equilibrium) theories of reversible chemical action is the extreme slowness with which the stain is released when water is the external medium.

(c) In face of these contradictions it is perhaps not surprising to find a more vitalistic view represented chiefly by the theory of "physiological

permeability" formulated by such a distinguished authority as HÖBER (6, 7, 8, 9) and supported by TROENDLE (28). It involves the idea, specifically formulated by the latter author, that the accumulation of dyes, or at least acid dyes, as of other substances which do not at all times freely enter the cell, is the result of work done by the living protoplasm. It is proposed in the following pages first of all to indicate briefly some evidence which is opposed to regarding such vital intervention as necessary and thereafter to demonstrate for a few particular cases the apparent nature of the storage mechanism.

Results

I. ACCUMULATION OF DYE IN CELLS WITHOUT "PHYSIOLOGICAL ACTIVITY"

Dye may accumulate in higher concentration inside than outside cells under conditions in which activity of the protoplasm can be ruled out.

When, for example, only the vacuolar membrane is functioning, the rest of the cell having been killed by iodine, eosin will slowly enter and accumulate in the sap slightly in excess of its external concentration. In cells of *Spirogyra* mounted in concentrated glycerine this condition has been maintained for weeks. It is difficult to regard the vacuolar film as being capable of doing work under such circumstances, though it does retain a certain degree of semipermeability, which, however, may be purely physical.

Cells which are beyond question totally dead may sometimes retain dye in the sap. When sections from leaves of *Anthericum*, in which the vacuole has become deeply stained by acid fuchsin, are treated with toxic concentration of acetic acid, the dye soon leaves the sap of the majority of the cells but is usually retained by a few of them in almost undiminished concentration for a considerable time. Such cells are, according to other criteria, quite dead, stained as to nuclei and chloroplasts, and unplasmolysable. The effect of attempting plasmolysis is to cause immediate escape of dye from the sap, and the same happens if, with a needle for example, mechanical pressure is applied to the cell. As to how these cells differ from the rest the suggestion may be made that either the tonoplastic or ectoplastic membrane is still intact and that mere coagulation is not sufficient to allow escape of the dye unless mechanical rupture, such as normally syneresis might produce, also ensues. At any rate it appears that a cell need not be alive to hold the dyestuff in its sap.

Even the cell wall, if the solution within it contains colloidal matter, may serve to retain dyes to which it is normally readily permeable. Concentration of dye has been observed to take place in the space between the protoplast and cell wall in occasional living plasmolysed cells of *Spirogyra* and also frequently in dead and moribund examples of such cells and of gametangia in the same plant. Dye, acid or basic, which enters in a few

minutes may take hours to wash out, though to all appearance in homogeneous solution. That the dye is held by colloidal matter is shown by precipitation of the latter with CaCl_2 , after which there is no retention of stain.

II. THE MECHANISM OF STORAGE IN LIVING CELLS

A. STAINING OF THE CELL SAP.—The usual loci of vital staining are granules and sap vacuoles. As regards the former it seems superfluous to postulate any other mechanism than a physical or chemical combination with the colloidal material of the body stained; as regards the sap the opinions, as has been pointed out, are many and conflicting. Therefore the following contribution to the problem, based upon a careful study of a few types rather than a wide survey either of plants or stains, and presenting as it does, some definite results at least for basic dyes, is not superfluous.

1. BASIC DYES.

(a) *Spirogyra*.—The intravital precipitates with basic dyes in *Spirogyra* have been studied especially by PFEFFER and VAN WISSELINGH (30). The facts may be briefly stated from the view of the present thesis. When *Spirogyra* is placed in dilute neutral red, methylene blue, thionin, Janus green, Bismarck brown, etc., the vacuole at first stains homogeneously, but, as dye accumulates, a precipitate forms which may hold all the visible color. According to VAN WISSELINGH it is colorless on its first appearance if the dye is applied as dilute as 1 in 500,000. This precipitate is located both in the vacuole and in the cytoplasm, originating in the latter at least by the staining and aggregation of granules visible without stain. While the precipitate usually appears granular, the aggregates having only a slight tendency toward plastic coalescence, it may under certain conditions run together of its own accord into droplets or even to a single drop in each cell, the cytoplasmic precipitate sometimes passing into the vacuole. Addition of weak alkali (ammonia) or an alkaloid (caffeine) also produces this flux of the colored precipitate and of any dye dispersed in the sap. In either case all the visible stain may be held by the drops and no more accumulate in the watery phase. The precipitate disperses again on addition of weak acetic acid. The substance in the sap which thus precipitates with the stain can also be induced, both by caffeine and by ammonia, to precipitate in similar but somewhat less striking fashion without staining. If the cells be now exposed to dilute stain, it is again the droplets which absorb it while the surrounding sap is not visibly colored. *It is evident therefore that concentration of dye in the sap as in the cytoplasm is dependent upon its combination with some colloidal material.* The exact nature of this substance is unknown. The reaction between the sap and caffeine or ammonia is given by PFEFFER as a test for tannin. The principal part of the pre-

precipitate is probably not tannin, however, but something with which it is combined or adsorbed, the nature of which will be discussed later. Even the presence of tannin is not essential for the formation of a droplet precipitate of basic stain with sap colloid, as will be shown in the case of other cells.

(b) *Allium*.—The epidermis of bulb scales was used. In this case basic dyes are stored in the sap without precipitation. The reactions to be described are best obtained after accumulation of the dye has approached saturation and only in cells which store it in high concentration, viz.: in the outer epidermis of the inner scales. In *Allium* no precipitate is usually produced by caffeine or ammonia alone, but when these reagents are added under the conditions of staining just mentioned, the development and fusion of droplets occurs as in *Spirogyra* but commonly in even more remarkable fashion. The homogeneous sap is suddenly transformed into a dense mass of tiny droplets which proceed turbulently to coalesce. The appearance is similar to that of the precipitation of oil from an alcoholic solution on addition of water or evaporation of the alcohol. The volume occupied by the precipitate relative to the rest of the sap is surprisingly large and indicates that the drops are largely hydrated.

Incidentally an interesting behavior, first noted by Professor F. E. LLOYD, of the globular precipitate in the sap of *Allium*, etc., offers an analogy to that of contractile vacuoles. In weak alkali the globules become vacuolated, swell and burst, discharging the content of their "vacuole," which like the watery sap is poorly stained. They then round off again, to repeat the process. The contractile vacuoles which can be experimentally induced to form in the cytoplasm and which may discharge to the exterior, appear to differ in no essential from the intra-vacuolar variety. Whether the latter is a purely physical model, or whether the sap shares vitality with the cytoplasm is a dilemma we need not here discuss.

The condensation of the dye-colloid combination in the sap is produced by ammonia before the color of the neutral red changes. Acidification with acetic acid causes the drops to disappear again, some dissolving gradually, others appearing to lose suddenly the bounding film over a part of their surface, with a gushing out of the whole content. There is evidence of a critical P_H range for condensation, excess of alkali producing an effect somewhat similar to that of acid. At any rate the large drops frequently burst, but the contents are scattered as very fine droplets instead of homogeneously as with acid. In *Allium* and *Spirogyra*, alkali tends to harden the drops or at least their periphery and this change interferes with dispersion; but in the guard cells of the stomata in this and other plants, in which the drops remain fluid in weak alkali, the dispersal is complete as will be presently described.

While it cannot be stated for *Allium* as it can for *Spirogyra* that practically all the adsorbed dye is contained in the precipitate, yet, in view of

the fact that alkali tends to release the stain from its combination, it may all have been held by the colloidal antecedent of the drops in the natural slightly acid condition of the sap.

(c) *Tradescantia zebrina*.—These studies were made on the guard cells of the stomata. All the epidermal cells of *Tradescantia* absorb basic dyes, which results in more or less precipitation when the accumulation has reached a certain stage. In the naturally colored cells the native pigment is included in the precipitate. In these, and better in the colorless cells adjoining the stomata, fusion of droplets occurs, while the reactions to penetrating acids and bases resemble those in *Allium*. The stomatal guard cells, as in other leaves examined, have a more powerful affinity for basic dyes and also present certain features of peculiar interests. As in the adjoining cells the dye-colloid combination seems to be near the P_H of maximum condensation. For, when sufficiently deeply stained, it condenses into a few vivid droplets occupying a large proportion of the vacuole while the rest of the sap becomes more or less colorless. Further observation indicates that more commonly it is the whole vacuole which thus breaks up into drops, the colorless environs being protoplasmic. The drops expand and coalesce to fill the vacuole if either a penetrating alkali (ammonia) or a penetrating acid (acetic) is added. The alkaline P_H which produces this effect is not injurious to the cell but the acid P_H is apt to be harmful if long continued.

Parenthetically, a fact may be noted which is very suggestive in connection with the mechanism of stomatal regulation, viz.: that the stomata close throughout the intermediate P_H range in which the drops condense, and open on either side of this when the drops expand and apparently exercise pressure. Opening and closing of the stomata follow the P_H changes of the sap in normal as well as in stained cells. Thus it is evident that when CO_2 is being utilized (in photosynthesis) and the P_H rises, the stomata will tend to open; when it is allowed to accumulate they will tend to close, unless, possibly, the acidity rises beyond the region of closure to that of opening on the acid side. Judged by the reaction of the external medium the point of maximum condensation appears to lie just on the acid side of neutrality. The color of neutral red in the sap is also red at this point, but this is a fallible guide to H-ion concentration if the stain is adsorbed.

In various other cells besides those described above, accumulation of basic dyes with or without the help of alkali, etc., has been observed to produce droplet formation. The natural pigment of colored cells containing tannin may also be included in the precipitate (VAN WISSELINGH). While on the other hand many examples could be mentioned of cells that store dye to a moderate extent without the ability to form a precipitate such as has been described, it does not follow that there is no colloidal combination in such cases, because the drop formation phenomenon is evidently the result of a rather critical combination of factors. Rather, from the cases in which

the colloidal combination can be proved essential, should we extend the probability to others.

(d) *Nature of the intra-vacuolar colloidal material with which basic dyes combine.*—PFEFFER regarded the precipitate which methylene blue produces in *Spirogyra* as simply a compound of the dye with tannin. VAN WISSELINGH emphatically denies this as well as the precipitating power of methylene blue *per se* on tannin *in vivo* or *in vitro*. The latter author has also investigated very fully the nature of the precipitate which alkalis, alkaloids, etc., produce in the same plant and critically rejects the view of PFEFFER and others that protein as well as tannin enters into it. Protein, he says, is absent from the sap of *Spirogyra*, and, if introduced, precipitates with the tannin, unaided by alkalis. At most the tannic acid can form only a very small fraction of so voluminous a precipitate even in *Spirogyra*, while in the onion epidermis in which the precipitate is equally great, tannin, if present at all, is in very minute quantity. In the stomata of *Tradescantia* the droplets occupy an even greater relative proportion of the sap without the presence of any detectable trace of tannin. It is perhaps significant that in these cells the drops do not superficially harden in alkali as they do in *Spirogyra* and *Allium*, suggesting that that phenomenon may be due to the development of a surface film, *e.g.*, of insoluble tannate. LLOYD (15, 16) has also shown that tannin in the idioblasts of persimmons, acorn, etc., is adsorbed by the colloidal material of the tannin mass.

In the writer's opinion the summation of properties of these precipitates points to a largely lipoidal composition. Their fluidity, high refractive index, reduction of osmic acid—which however is also a property of tannins—and particularly their powerful affinity for the stains that are adsorbed by lipoids all point in this direction. Moreover, as recently described, normal cells of *Spirogyra*, and, it may be added, of *Allium*, *Anthericum*, and probably other cells, contain both in sap and cytoplasm bodies which simulate lecithin in the development of "myelin forms," and which when rounded off are apparently identical with the droplets produced under the action of bases and basic dyes. Bearing on the character of the drops is the fact that within large drops smaller ones may form. In view of this behavior and of the relatively large volume occupied by the precipitate as a whole, the drops must internally at least be no more than an aqueous sol. Their sharp demarcation from the sap or cytoplasm may be explained on the assumption of the development of a superficial mono- or bi-molecular layer—as is postulated also in explanation of the "myelin forms" of lecithin by LEATHES (14). It may be, as suggested above, that organic acids, *e.g.*, tannic acid, as well as the lipoids themselves, enter into the formation of these boundary films. Their sudden disappearance in acid, and their hardening in alkali, support this view.

(e) *Relation of accumulation of basic dye to the P_H of the sap.*—The relation between the P_H of the external medium and the P_H of the sap could influence the relative concentration of dye at equilibrium if either its ions or its undissociated molecules are unable to pass the plasma membrane. There is plenty of evidence, OSTERHOUT (21), McCUTCHEON and LUCKE (18), IRWIN (12, 13), that undissociated molecules penetrate the more readily, but the absolute impenetrability of the ions, which is demanded if the state of equilibrium is to be determined thereby, is contrary to other evidence, VAN SLYKE, WU, MACLEAN (29), BROOKS (2, 3), SCARTH (26). Nor does variation of the external P_H alone, while it greatly influences rate of penetration, have any profound effect on the total accumulation of stain. The rate of adsorption of a basic dye falls as the external P_H falls, but even if the latter is made lower than that of the sap, the final accumulation of dye is so little affected that it proves the P_H ratio to be of very minor importance in this respect.

Variation of the internal P_H has, however, a notable effect on storage, whatever the external P_H may be. Within limits as shown by McCUTCHEON and LUCKE, and quantitatively by IRWIN, the rate of absorption falls if the P_H of the sap is increased above normal. The effect of reducing below normal is not recorded, but from the sequel a reduction rather than an increase may be expected. For as PFEFFER pointed out, vitally stained filaments of *Spirogyra* rapidly lose their stain if exposed to a penetrating acid such as citric. The stain, if concentrated in granules, first leaves these to color the sap and then diffuses out of the cells. Other varieties of cell above mentioned also lose a certain proportion of their accumulated stain without irreversible injury when acetic acid is added to the medium. The amount of basic dye that the cells can hold in balance with a given external concentration is evidently lessened as the sap is acidified from a P_H of about 5.5 (distilled water).

The interaction of variables is more complicated when we consider the staining relations on the alkaline side of neutrality. Penetration of alkali (ammonia) causes a certain loss—slight as compared with acid—of stain from the cells to the medium. (In the case of neutral red the original red color is of course restored by acidification before making comparison with a control). Similarly the guard cells of the stomata of *Tradescantia* are found to accumulate more neutral red after the epidermis has been exposed 24 to 48 hours to a weak solution of distilled water (P_H 5.5) than if enough ammonia is added to render the solution slightly alkaline. Possibly the added base may compete chemically with the color base, e.g., for combination with the organic acids of the sap, and we know that tannic acid when it is present, as in *Spirogyra*, enters into the precipitate produced by weak bases and by basic dyes.

It is to be noted, however, that the dyes produce no precipitate with tannic acid *in vitro*, nor does ammonia in the low concentration in which it is active *in vivo*, so that at least a third constituent, viz.: the colloid matter in the sap is essential. The exact nature of the combination between the dye and this colloid with lipid properties must be uncertain in absence of definite knowledge of the possible part played by organic acids of the sap. The union which takes place between basic dye and hydrated lipid alone is known to be one of adsorption from the work of LOEWE (17). But should the dye first combine with a lipid-soluble acid the compound might pass into solution. Such is NIRENSTEIN's theory of dye absorption. He found, working with *Paramoecium*, that the relative extent to which the various basic dyes are taken up by that organism corresponds with that of their absorption from water by a mixture of olive oil and oleic acid, the addition of a fat-soluble organic base, diamylamine, extending the correspondence to acid dyes without interfering with the uptake of basic.

The relation between the P_H of the sap and the retention of dye appears to favor NIRENSTEIN's rather than a pure adsorption theory inasmuch as the presence of alkali ought to depress accumulation of basic dye if that were due to acid combination, and increase it if due to adsorption by an amphoteric colloid. Actually the former tends to happen.

Possibly the exact mode of combination varies in different cells but the common feature on which stress is laid is that the dye may combine with a colloid having lipid properties and be stored simply in virtue of this combination.

2. ACID DYES.

(a) *Spirogyra*.—It was desired to find out if the colloidal material which absorbs basic dyes has under any conditions an affinity for acid dyes also. The latter do not enter normal *Spirogyra* cells in appreciable quantity even after several days' exposure, but can be induced to do so rapidly by treating the cells with a strongly hypertonic solution (*e.g.*, molar sugar solution) or by successively plasmolysing and deplasmolysing in presence of the stain. The details and significance of this phenomenon are to be discussed elsewhere, SCARTH (27). It was found that cells which have absorbed dye in this way do not react to give the usual precipitate with ammonia or caffeine, hence the precipitate had to be first produced and the filaments thereafter stained by the above method—still in presence of caffeine to maintain the condensation. Under these conditions the absorption of acid dyes (eosin and acid fuchsin) by the drop is either slight or imperceptible.

But on acidification with acetic or citric acid they acquire almost as strong an affinity for acid dyes as they ordinarily possess for basic. Owing to their tendency to disperse in acid the drops have to be large to begin

with in order that they may endure until stained. Sufficiently prolonged treatment with caffeine and selection of suitable species of *Spirogyra* can procure this condition. The inference from the staining property of the drops in relation to H-ion concentration is that they consist of an ampholyte, and probably lipoids as well as proteins have this property. On the acid side of its isoelectric point this colloid takes up only acid dyes; on the alkaline side only basic to any marked extent. From the staining relation one would suppose that the isoelectric point is more acid than the normal sap and the P_H of greatest condensation. It may be recalled that frequently the isoelectric point and that of maximum aggregation of a colloid do not entirely coincide.

While high acidity of the sap causes staining by acid dye, once it has penetrated, it does not appear to induce an appreciably greater penetration in the case of *Spirogyra*. No visible staining with acid fuchsin takes place in presence of citric acid, at least while the cells remain normal. The failure of acid dyes to enter is apparently a function of permeability rather than storage. The study was therefore extended to cells which under normal conditions are freely permeable to dyes of this class.

(b) *Anthericum*.—The leaf tissues were used for this study. Cells of the tissue of many higher plants absorb acid dyes quite readily, whether brought to them by the transpiration current or by diffusion into cut sections. The sections have to be thick as the outer cells usually die quickly. In some cases the dye never surpasses or perhaps may never equal (COLLANDER) in concentration that of the external medium and in such cases it quickly washes out in water; but when it accumulates—as in *Anthericum*—it scarcely fades until the cells die, which may not be for several days. Acid fuchsin was most commonly used, being readily diffusible, deeply colored and relatively non-toxic; but its property of losing color in alkali makes it necessary in experiments with ammonia to acidify with acetic acid before making color comparisons.

No direct evidence of colloidal combination could be obtained; but neither with basic dyes, although they are fairly strongly stored, do we get any such precipitate with caffeine, etc., as in the other cases described. A slight flocculation of the sap with acid in some of the cells after death was the only visible evidence of the existence of organic matter in the central vacuole. The difficulty of washing out the dye while the cell is alive is admittedly open to other explanations than adsorption, but the cases of retention by dead cells quoted earlier are more difficult to explain on other grounds, as is RUHLAND's observation that acid dyes take time to enter into a state of "storage."

The relation of storage to H-ion concentration throws little light on the mechanism. In *Spirogyra* the sap had to be made decidedly acid for the

droplets to stain appreciably with acid dyes but here the reaction is normally not far from neutral. Yet the dye accumulates, nor does there seem to be much difference in the resistance to washing whether tap water alone, water with a trace of acetic acid, or with a trace of ammonia is used. If anything, alkali tends to release the stain. The experiment cannot be extended very far in the alkaline direction, however, owing to the low viability of the cells in such a medium. These relations demonstrate that if adsorption should be the mechanism of storage it is of a different type from that obtaining with basic dyes which fail to be stored on the acid side of the isoelectric point. The comparative indifference to P_H in the case of acid dyes indicates that adsorption, if it exists, is "mechanical," as when proteins adsorb such dyes on the alkaline side of the isoelectric point.

B. STAINING OF THE PROTOPLASM.—

1. BASIC DYES.—The behavior in the cytoplasm has already been described incidentally in dealing with accumulation in the sap. In *Spirogyra* for example it is easy to observe the accumulation of the various basic stains in certain pale cytoplasmic granules. These include the so-called mitochondria but there appears to be a gradation of affinity for stain among the cytoplasmic granules rather than hard and fast distinctions. As they stain, the particles aggregate, sometimes forming branching, tree-like groups, sometimes tending to coalesce as already described. The behavior of these cytoplasmic aggregates differs in no essential from that of the precipitate which forms in the sap, and the predominantly lipid composition of the mitochondria at least is generally admitted. The markedly amphoteric behavior of the granules has already been mentioned.

Usually no other structure of the cytoplasmic material stains in life, but in one species of *Spirogyra* instances of staining by neutral red of the lining of the sap vacuole have been observed. The stained layer extends over the surface of the cytoplasmic strands and nuclear investment. On treatment with caffeine the stained tonoplast behaves like the stained precipitate, *i.e.*, it contracts as in abnormal plasmolysis, at first squarely but later as a rule rounding off to a large sphere. This phenomenon is of significance to theories of the composition of protoplasmic membranes.

2. ACID DYES.—No protoplasmic staining appears to take place in normal cells of *Anthericum* which are naturally permeable to acid dyes, but those of *Spirogyra*, when rendered artificially permeable by strongly hypertonic sugar solution, become stained while the cells are still alive. In the early stages when the nucleus alone is stained, and not too deeply, the cells may recover from the treatment. The stain is slow in washing from the nucleus as we might expect if it is adsorbed.

C. STAINING OF THE CELL WALL.—Inclusion of reference to the cell wall in a treatise on vital staining is justified by the facts which have been ascer-

tained, which show that the vital and the post mortem staining of the wall are not the same. And if the cell wall changes at death the inference is that it is an integral part of the living cell and itself alive.

The cell walls of living cells are much less readily stained by basic dyes (thionin, methylene blue, neutral red, etc.) than are those of dead cells. Below a certain critical concentration of dye there is no visible staining of the wall in the living cell no matter how prolonged the exposure, while with far lower concentrations there may be deep staining after death. With respect to *Spirogyra* and the dye thionin, the critical concentration in life is about $M/10,000$, though it varies for different species, filaments, and even cells of a filament. Cells which have been killed by vapor of ether or of iodine, however, acquire vividly blue walls when left overnight in more dilute solutions ($M/20,000$ or less) of the dye. The same is usually true of cells which have died accidentally, though on the other hand certain methods of causing death, as for example by hot water or steam, may greatly weaken this affinity for the stain, possibly through leaching the lipoids from the cell wall. Dilute acid and polyvalent cations also substitute acidophil for basophil qualities in the wall as in other amphoteric substances.

In higher concentrations of dye ($M/4,000$ and upward) which stain the walls whether the material is alive or not, the addition of CaCl_2 or salts of other non-toxic bivalent or trivalent cations reduces or prevents the staining by basic dye to a much greater degree in the live material than in the dead. Mere contact with the living protoplast, as plasmolysis experiments demonstrate, cannot account for these differences of staining of the wall in life and death respectively.

It is clear that the staining quality of the cell walls in *Spirogyra*, for example, is different from that of say filter paper or cotton fibre. For one thing the affinity of the former for dilute dye is greater and for another the amphoteric quality is far more pronounced. In this respect the resemblance is closer to the lipid granules (mitochondria) of the cytoplasm than to the celluloses and pectoses of the wall. In view of HANSTEEN-CRANNER's chemical analyses of cell wall substance, the conclusion is legitimate that staining of the wall by basic dye is in part a function of the interpenetrating lipid. The fact that certain modes of killing the cell increase the staining capacity of the wall may be due to the lipid substance tending to coagulate or become dehydrated in death like the protoplasm itself.

Summary

BASIC DYES.—It is shown in most of the cases studied, especially if there is deep staining, that basic dyes combine with some colloidal material in the sap as well as in the cytoplasm and that when this condenses it carries all

or most of the dye with it and leaves the cell incapable of further vital staining.

The affinity of this material for basic dyes is greatest when the cell sap has a slightly acid reaction, apparently between P_H 5 and P_H 6. If rendered more acid, the dye leaches out of the precipitate and out of the cells and the precipitate acquires an affinity for acid dyes which it does not possess on the alkaline side of this region. There is also a slight loss of basic dye if the sap is made alkaline.

The drops of colloidal material disappear by expansion or dispersion if the sap is acidified and, in some cells, also if it is made decidedly alkaline; but in others hardening of the periphery seems to interfere with this process. The P_H region of maximum condensation appears to be higher than the isoelectric point as indicated by staining relations.

The colloidal substance thus behaves as an ampholyte and its other physical properties suggest that it is partly hydrated lipid, enveloped probably by a mono- or bi-molecular film.

Since a combination of factors is necessary to cause the colloid to condense into drops, a negative result in particular cases does not prove the absence of colloid in such cases. The nature of the mechanism of accumulation in cells which stain without exhibiting the precipitation phenomenon therefore remains an open question, with the presumption by analogy in favor of colloid combination. As to the source of attraction between basic dye and colloid it is shown that NIRENSTEIN'S theory of intermediate combination of dye with a "lipoid soluble" acid, or a lipoid adsorbable one like tannic acid, fits the facts to a large extent, but that direct adsorption may also be a factor.

The predominant part played by lipoid in the vital staining by basic dyes of the sap and cytoplasm appears to extend in some measure to the cell wall also.

ACID DYES.—While the ability of the normally basophil colloidal material to take up acid dyes when the sap is acidified points to the possibility of their adsorption in the cell, the actual mechanism of storage by the cells of *Anthericum* which normally accumulate such dyes remains undecided.

McGILL UNIVERSITY,
MONTREAL, CANADA

LITERATURE CITED

1. BETHE, A. Der Einfluss der H-Ionenkonzentration auf die Permeabilität toter Membranen, auf die Adsorption in Eiweisslösungen und auf den Stoffaustausch der Zellen und Gewebe. *Biochem. Zeitschr.* **127**: 18-33. 1922. (See citation no. 4 for earlier papers by BETHE.)

2. BROOKS, M. M. The penetration of cations into living cells. Jour. Gen. Physiol. **4**: 347-349. 1922.
3. ————. Penetration into *Valonia* of oxidation-reduction indicators; estimation of the reduction-potential of the sap. Proc. Soc. Exp. Biol. and Med. **23**: 265-266. 1926.
4. COLLANDER, R. Über die Permeabilität pflanzlichen Protoplasten für Sulfosäurefarbstoffe. Jahrb. Wiss. Bot. **60**: 354-410. 1921.
5. HOAGLAND, D. R., and DAVIS, A. R. The composition of the cell sap of the plant in relation to the absorption of ions. Jour. Gen. Physiol. **5**: 629-646. 1923.
6. HÖBER, R. Eine Methode, die elektrische Leitfähigkeit im Innern von Zellen zu Messen. Archiv ges. Physiol. **133**: 237-253. 1910.
7. ————. Ein zweites Verfahren, die Leitfähigkeit im Innern von Zellen zu Messen. Archiv ges. Physiol. **148**: 189-221. 1912.
8. ————. Messungen der inneren Leitfähigkeit von Zellen. Archiv ges. Physiol. **150**: 15-45. 1913.
9. ————. Physikalische Chemie der Zelle und der Gewebe. 5th ed. Leipzig. 1922.
10. IRWIN, MARIAN. The behavior of chlorides in the cell sap of *Nitella*. Jour. Gen. Physiol. **5**: 427-428. 1923.
11. ————. On the accumulation of dye in *Nitella*. Jour. Gen. Physiol. **8**: 147-182. 1925.
12. ————. Accumulation of brilliant cresyl blue in the sap of living cells of *Nitella* in the presence of NH_3 . Jour. Gen. Physiol. **9**: 235-253. 1925.
13. ————. Mechanism of the accumulation of dye in *Nitella* on the basis of the entrance of the dye as undissociated molecules. Jour. Gen. Physiol. **9**: 561-573. 1926.
14. LEATHES, J. B. Rôle of fats in vital phenomena. Lancet **1**: 803-807, 853-856, 957-962, 1019-1022. 1926.
15. LLOYD, F. E. The behavior of tannin in persimmons, with some notes on ripening. Plant World **14**: 1-14. 1911.
16. ————. The association of tannin with an emulsion colloid in the acorn. Johns Hopkins Univ. Circ. 15-18. 1912.
17. LOEWE, S. Beziehungen der Lipoide zu den Farbstoffen. Biochem. Zeitschr. **42**: 150-189. 1912.
18. McCUTCHEON, M., and LUCKE, B. The mechanism of vital staining with basic dyes. Jour. Gen. Physiol. **6**: 501-507. 1924.
19. NIRENSTEIN, M. Über das wesen der Vitalfärbung. Archiv ges. Physiol. **179**: 233-337. 1920.
20. OSTERHOUT, W. J. V. Some aspects of selective absorption. Jour. Gen. Physiol. **5**: 225-230. 1922.

21. ————. Is living protoplasm permeable to ions? Jour. Gen. Physiol. **8**: 131–146. 1925.
22. ————, and DORCAS, M. J. The penetration of CO₂ into living protoplasm. Jour. Gen. Physiol. **9**: 255–267. 1925.
23. RUHLAND, W. Studien über die Aufnahme von Kolloiden durch die pflanzliche Plasmahaut. Jahrb. Wiss. Bot. **51**: 376–431. 1912.
24. ————. Zur Kritik der Lipoid- und der Ultrafiltertheorie der Plasmahaut nebst Beobachtungen über die Bedeutung der elektrischen Ladung der Kolloide für ihre Vitalaufnahme. Biochem. Zeitschr. **54**: 59–77. 1913.
25. ————. Zur Kenntnis der Rolle des elektrischen Ladungssinnes bei der Kolloidaufnahme durch die Plasmahaut. Ber. D. Bot. Ges. **31**: 304–310. 1913.
26. SCARTH, G. W. The penetration of cations into living protoplasm. Amer. Jour. Bot. **12**: 133–148. 1925.
27. ————. The influence of external osmotic pressure and of disturbance of the cell surface on the permeability of *Spirogyra* for acid dyes. Protoplasma **1**: no. 2. 1926. In press.
28. TROENDLE, A. Über die Permeabilität des lebenden Protoplasma für einige Salze. Arch. Sci. Phys. Genève **45**: 38–54, 117–132. 1918.
29. VAN SLYKE, D., WU, H., and MACLEAN, F. C. Factors controlling the electrolyte and water distribution in the blood. Jour. Biol. Chem. **56**: 765–849. 1923.
30. VAN WISSELINGH, C. On intravital precipitates. Rec. Trav. bot. Neerl. **11**: 14–36. 1914.

EVIDENCE ON THE INDISPENSABLE NATURE OF ZINC AND BORON FOR HIGHER GREEN PLANTS

A. L. SOMMER AND C. B. LIPMAN

(WITH THIRTEEN FIGURES)

Introduction

For more than a century, plant physiologists contented themselves with the assumption that only ten of the eighty odd chemical elements known are essential and indispensable to the life and growth of the chlorophyllous plants. With one important modification, the list of these ten elements was that furnished by THEODORE DE SAUSSURE in his epoch-making book published in 1804. The one important modification of the list cited came about through the celebrated investigations of KNOP about one-half century later who showed iron to be essential, and silicon, as he thought, non-essential, thus making a substitution of iron for silicon in DE SAUSSURE's list. For more than half a century after KNOP's investigations, the slightly modified DE SAUSSURE list of the essential chemical elements held the center of the stage in that field in all textbooks and reference books on plant physiology and related subjects. From our point of vantage, it seems singular that the assumption under review should have been made without further inquiry in the face of the abundant evidence available on the composition of the ash of plants. To be sure, it is obvious that the mere presence of an element in a plant can constitute of itself very flimsy evidence of its indispensable nature to the plant, but it is none the less a circumstance which cannot be waved aside without investigation. Other circumstances than the mere inability of physiologists to apprehend the problem in the proper light conspired to maintain it in the doldrums for so long a period. These were the seemingly inherited human characteristic of ignoring imponderables, the crudity of methods employed for growing plants, and the impurity of the so-called "chemically pure" chemicals. Fresh impetus for the study of this problem was not forthcoming until methods of work became much improved, and certain experiments indicated that some other chemical elements than those on the "preferred" list could improve the growth of plants and serve as "stimulants," and, further, until certain brilliant discoveries in animal physiology introduced the concept of the hormone and the vitamins into our philosophy and theory of general physiology. Even these occurrences have impressed only a few plant physiologists with the real nature of the situation, which obtains on the subject of the indispensa-

ble chemical elements for plants. Only one of these physiologists, MAZÉ (2, 3, 4), has taken a comprehensive view of the problem until within the last eight or ten years. That distinguished student of plant physiology and chemistry has discussed, in a number of important contributions, the broad problem involved, and has shown by the use of new methods of work the strong probability that several chemical elements besides those theretofore considered essential to green plants might be proved indispensable. His early experiments impressed MAZÉ so much as to make him assume that with very pure chemicals it is impossible to grow plants in culture solution of the usual composition without the addition of small amounts of several of the rarer elements found in the ash of plants. He had very pure chemicals prepared especially for his use and in making his culture solutions, he added very small quantities of such elements as zinc, aluminum, chlorine, boron and others, to those which have long been considered essential to plants. He found that in the absence of these additional elements, plants would not live in culture solutions furnishing only the traditionally important elements of KNOP'S media. These experiments led him to conclude that zinc, aluminum, chlorine, silicon, boron, iodine and perhaps several other elements, are essential to the life and growth of green plants. Since we are considering in this paper studies on zinc and boron only, we need not discuss MAZÉ'S work on the other essential elements until our further studies on other elements are published. Of the two elements under discussion here, it may be noted, however, that MAZÉ has furnished evidence of the indispensable nature of zinc for maize. Since he grew very few plants in his cultures, and since he worked with only one type of higher green plant, his results required confirmation. Such confirmation is furnished in our own results, obtained under better controlled conditions than those characteristic of MAZÉ'S experiments.

With MAZÉ'S published results as a stimulus, a number of investigators have attempted, within the last six to eight years, to obtain more conclusive and final evidence respecting the dependence or non-dependence of green plants on each of several of the more common elements found in plant ash. In general, these investigators questioned the correctness of MAZÉ'S views, but two or three of them, quite independently of one another, have recently furnished incontrovertible evidence that he is right, as regards general principles. These may now be reviewed briefly. MCHARGUE (5, 6, 7) has furnished indisputable evidence on the indispensable nature of manganese to green plants. He used purified chemicals in very pure sand and solution cultures as media. With a considerable variety of plants, he showed that they could grow for varying periods without manganese other than that present in the seed and the impurities of the medium, but that sooner or

later the plants would die or fail to mature unless manganese was added to the medium. Owing to the fact that a peculiar yellowing of the leaves became apparent in the plants growing with a deficient supply of manganese, MCHARGUE has concluded that the element in question performs some important function in the synthesis of the chlorophyll molecule. This is, of course, a hypothesis, but there can be no question, in view of MCHARGUE's evidence, and regardless of his hypothesis, that manganese is indispensable to the green plant.



FIG. 1. Sunflowers grown with and without boron (31 days). Upper row, with $\frac{1}{2}$ ppm. boron in the medium; lower row without boron.

In 1923, WARINGTON (8) furnished experimental evidence to the effect that the Windsor bean will not grow in culture solutions unless small quantities of boron are added to the usual culture solution. Following WARINGTON's striking evidence, BRECHLEY and THORNTON (1) have attempted to determine the cause of the influence of boron in the case of the Windsor bean and give experimental evidence which they interpret as indicative of the indispensable nature of boron to the legume bacteria growing on the roots of the Windsor bean, which require that element in the synthesis of organic nitrogen compounds with nitrogen which they obtain from the air. Since WARINGTON obtained evidence on the indispensable nature of boron

to one kind of plant only, the evidence for the establishment of a general need for boron by green plants was lacking, and instead of attempting to throw further light on that important feature of the problem, BRENCHELEY and THORNTON assumed that there was some specific need in the Windsor bean which boron is capable of supplying, thus overlooking its possible essential nature to other plants. Moreover, their results, which are cited



FIG. 2. Recovery of sunflowers on addition of boron to a boron-free medium. Left, one mg. ($\frac{1}{2}$ ppm.) boron as H_3BO_3 added to the medium after 31 days; right, no boron added.

above, have made them stray even further from the central problem, because of certain possible evidence in their results connecting the needs of *B. radicola* instead of its host with the boron problem.

During the last five years, the authors of the present paper have been engaged on some researches intended to test the views and experimental

evidence of MAZÉ, which are cited above and with which many physiologists have disagreed. Most of our efforts have centered about the elements silicon, aluminum, and chlorine. By the use of technique which is different and in our opinion superior for the purpose, to that used by other investigators of such problems, we have succeeded in showing, in spite of unusual difficulties involved in the study of the elements just named, that there is a strong probability of their being essential to green plants, though our evidence still lacks the finality which further experiments will probably furnish. One part of these experiments is described in a paper by A. L. SOMMER which is now in press and will soon appear in the UNIVERSITY OF CALIFORNIA Publications in Agricultural Sciences. While further studies on silicon, aluminum, and chlorine are in progress, we deemed it wise to

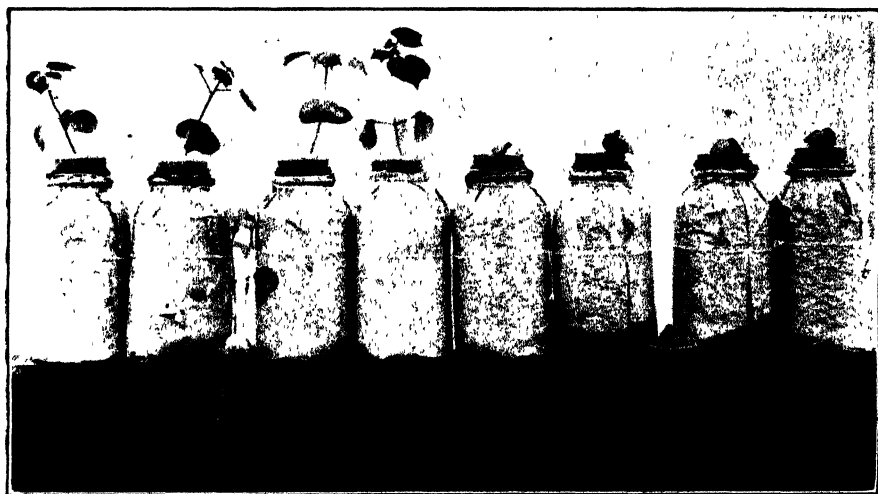


FIG. 3. Cotton grown with and without boron (29 days). Left, with boron; right, without boron.

test in the last few months the partial evidence obtained by WARINGTON on boron, which is described above, and to determine whether or not zinc is essential to green plants, as MAZÉ has claimed it to be, and as mycologists have shown it to be for certain physiological processes in fungi. The results of these two series of supplementary experiments have been so striking and conclusive that we hasten to give them to the scientific world before our earlier experiments on other chemical elements have fully matured.

Technique of the experiments

There are no experiments involving the use of the culture solution method which are so exacting as regards technique as those aiming at the determi-

nation of whether or not a chemical element like boron or zinc is essential to the life and growth of the green plant. For that reason, we deem it highly important to furnish in more or less detail our methods of work.



FIG. 4. Barley grown with and without boron (117 days). Upper row with boron; lower row without boron.

GREENHOUSE

Our plants were grown in a small greenhouse constructed within a larger greenhouse, for our purposes. This small greenhouse has a smooth cement floor which is kept as free from dust as possible. All ventilators are

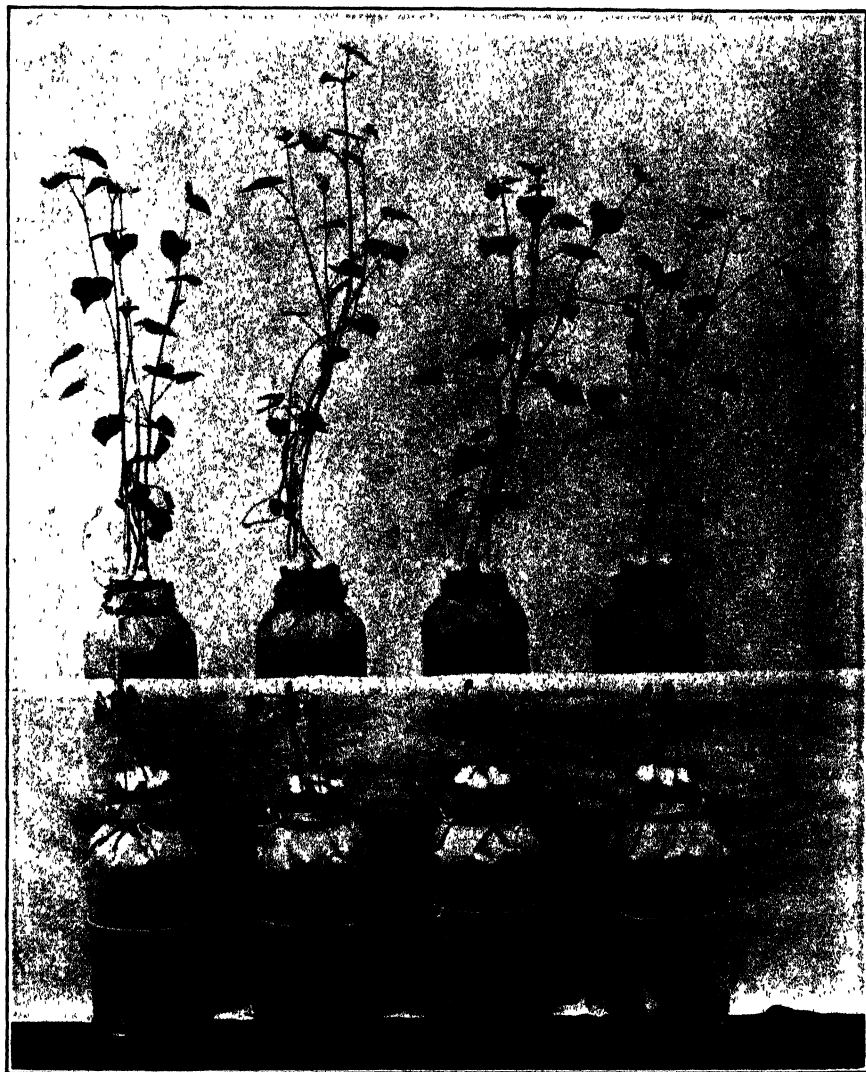


FIG. 5. Buckwheat grown with and without boron (28 days). Upper row with boron; lower row without boron.

screened so as to catch most, if not all, of the dust which might otherwise enter the compartment. A large fan attached to the compartment supplies a constant movement of air and obviates too high a temperature. A dust-free atmosphere has been found, through many disappointing experiences, to be essential to the success of the experiments.

COMPOSITION OF CULTURE SOLUTIONS

The following culture solution was employed for the early experiments on the boron problem:

KNO ₃	800 ppm.	Mn (as MnSO ₄)	1.5 ppm.
KH ₂ PO ₄	150 ppm.	Al (as Al ₂ (SO ₄) ₃)	0.5 ppm.
MgSO ₄ · 7H ₂ O	500 ppm.	NaCl	12.7 ppm.

300 cc. of saturated solution of CaSO₄ per liter was added to culture solutions, and FeSO₄ was added in small quantities to each culture as needed.



FIG. 6. Castor beans grown with and without boron (30 days). Upper row with boron; lower row without boron.

In later experiments on the boron problem and in those on the zinc problem, additions of $\frac{1}{4}$ ppm. of Cu,* F, and I were made as supplementary to the foregoing substances. These were found particularly important in the cultures grown in Pyrex containers. In the case of the boron problem, $\frac{1}{2}$ ppm. of zinc was added to all cultures. In the case of the zinc problem, $\frac{1}{2}$ ppm. of boron was added to all cultures, and also an excess of highly purified SiO₂, since Pyrex containers were used throughout and there was, therefore, the possibility of a shortage of silica in those experimental series.

* Since this was written, other experiments have shown that such concentrations of copper may be appreciably toxic for some plants. Further information in this regard will be given in a later paper.



FIG. 7. Flax grown with and without boron (26 days). Left, with boron; right, without boron.

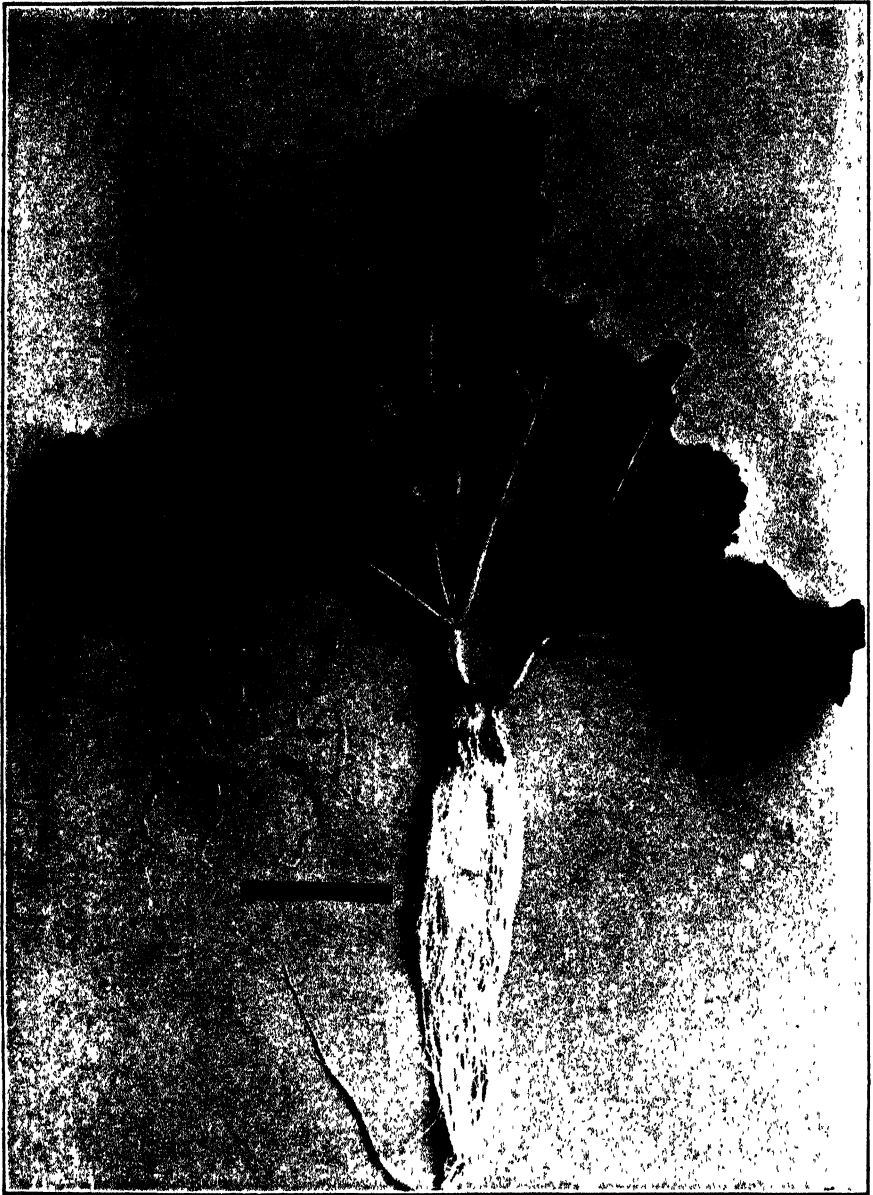


FIG. 8. Mustard grown with and without boron (66 days). Large plant with boron; small plants without boron.

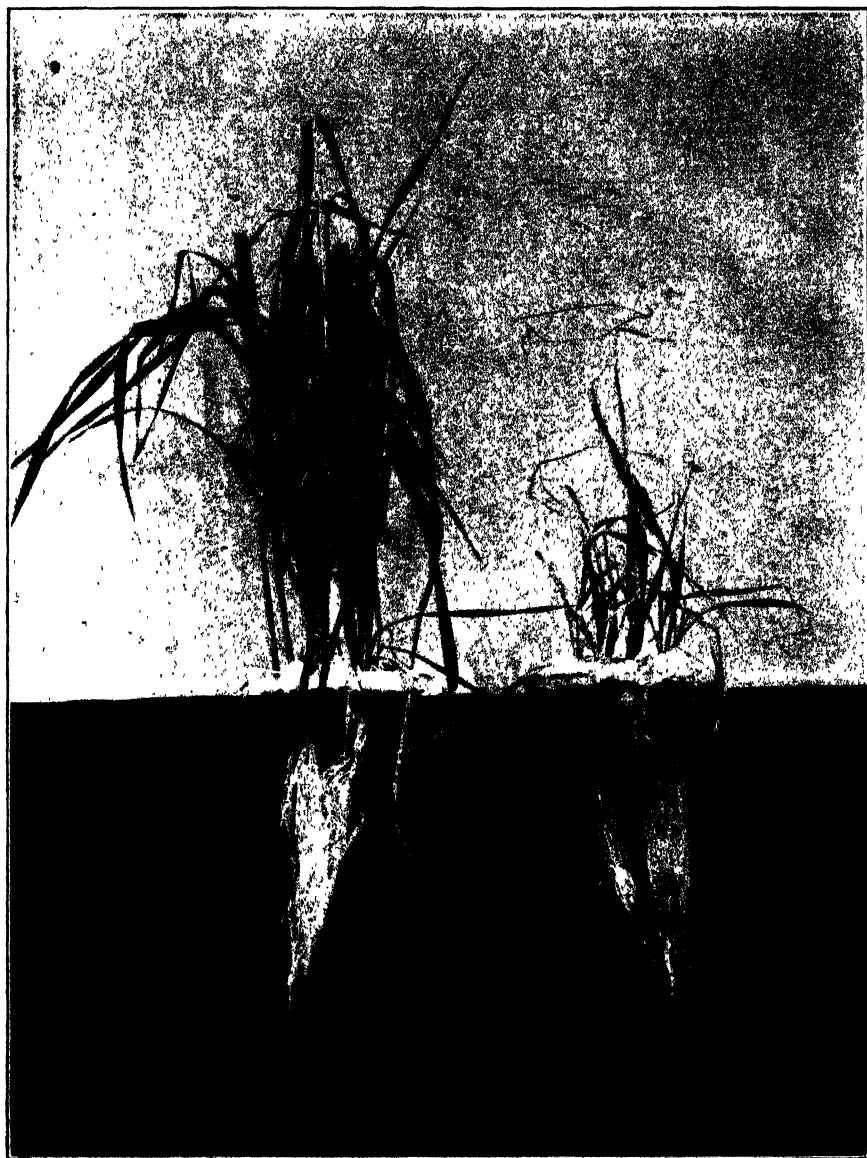


FIG. 9. Barley grown with and without zinc (51 days). Left with zinc; right without zinc.

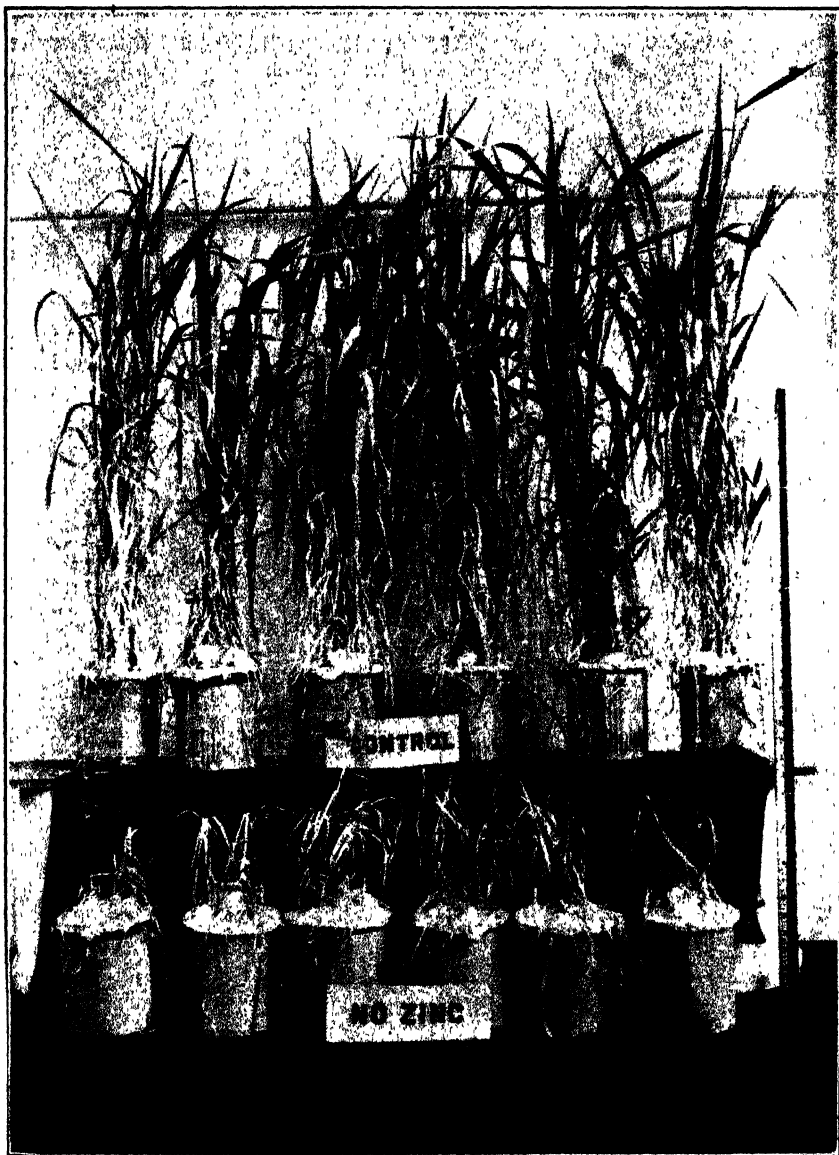


FIG. 10. Barley grown with and without zinc (118 days). Upper row with zinc; lower row without zinc.

CONTAINERS

Two kinds of containers were employed, depending on the element studied. In the case of boron, it was found unnecessary to use hard glass, since the amount of boron dissolved from the glass by the culture solution was relatively insignificant. For that reason, the ordinary Mason fruit jar, which had been thoroughly cleaned, was used in the boron series. In the zinc series, however, it was found to be absolutely necessary to use hard glass containers, since a considerable amount of zinc is dissolved out of the glass of the Mason jars by the culture solution, which would defeat the purposes of the experiment. For that reason, we used Pyrex beakers of

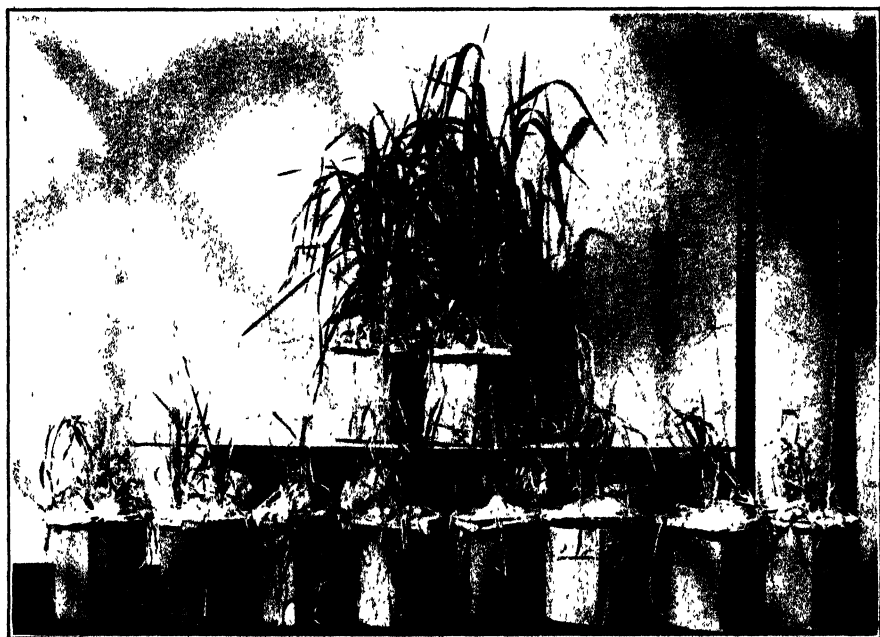


FIG. 11. Recovery of barley on addition of zinc to zinc-free medium. Upper row received .5 mg. ($\frac{1}{2}$ ppm.) Zn as ZnSO_4 . Added after 69 days of growth without zinc. Lower row, no zinc added.

one- and two-liter capacity in the zinc series. In the Mason jars, the usual paraffined corks were employed for holding the plants. For the Pyrex beakers, we made up plaster of Paris covers for the same purpose and paraffined them for protection of the solution and the plants.

WATER

The water used for preparing the culture solutions was specially double-distilled and protected from dust and fumes. For the researches with zinc,

the water was distilled into and stored in Pyrex flasks. Containers of ordinary glass were used for the water for the boron studies, because Pyrex is a boro-silicate.

CHEMICALS EMPLOYED

The salts used were those purified or specially prepared for the aluminum and chlorine studies, and were not tested for zinc or boron, because the physiological test made in these experiments was considered sufficient.



FIG. 12. Sunflowers grown with and without zinc, 79 days. Upper row with zinc; lower row without zinc.

AMOUNTS OF ZINC AND BORON USED

Preliminary tests showed that the addition of $\frac{1}{2}$ ppm. of boron, or of zinc, was adequate for the first several weeks of growth, and in some cases for the whole life period of the plant. The plants in the zinc experiment which received that element had only 0.0005 gm. per culture of four plants for the first two and one-half months of their growth, as shown in the photographs, figs. 1-13, accompanying this paper.



FIG. 13. Sunflowers grown with and without zinc, 79 days. Left with zinc; right without zinc.

KINDS OF PLANTS STUDIED

In the experiments with boron, we studied the Windsor bean, buckwheat, flax, mustard, castor bean, cotton, and barley. In the experiments with zinc, we studied sunflower (dwarf) and barley. The seeds were germinated in a solution of purified CaSO_4 . Wherever possible, the seed was cut off the seedling within a few days after germination, so as to deprive the seedling of as much as possible of the stored material in the seed.

Results

Since some of the plants would not grow at all, or only very slightly without additions of either of the elements tested, we are not furnishing data on the dry weights produced, but are giving, instead, photographs of certain stages in the growth of the plants which constitute much more striking evidence. In the figures which accompany the text, there will be found ready evidence of the results obtained in these experiments. These figures furnish such clear-cut answers to the questions at issue that much discussion is superfluous. It may be stated that the effects of the lack or presence of boron or zinc in the culture solution manifest themselves in the early stages of the growth period, and, in some cases, practically no growth is made by the seedling in the absence of the required element in the culture solution. Unlike the results obtained by McHARGUE in his studies on manganese, our findings indicate that in the cases of boron and zinc, there is not only a need for the element sometime in the cycle of growth, but it is needed from the very start. It is, of course, possible that that might also be the case with manganese if culture solutions were employed and made up of very pure salts, such as we used, and if the seed was removed from the seedling soon after germination. This may have been a factor in WARINGTON'S failure to show the need of boron in barley.

Our evidence renders it clear that boron and zinc are absolutely essential elements to the life and growth of certain higher green plants, and probably for all of them. As regards boron, the experiments show that WARINGTON saw only part of the picture. There is nothing peculiar about the Windsor bean in that respect, except possibly the quantity of boron needed. The need for boron seems to be general for higher green plants, and that may be stated as an empirical law. Moreover, it is entirely unnecessary to seek adventitious and more cryptic reasons for accounting for the relations of boron to the Windsor bean as BRENCHLEY and THORNTON have done. Irrespective of the needs of *B. radicola* for its synthetic processes, green plants in general require boron for the maintenance of the integrity of their cells. Our experiments prove conclusively, therefore, what was indicated by WARINGTON'S experiments, that boron is indispensable to the life and

growth of higher green plants, or at least for many of them. As regards zinc, we furnish indisputable evidence of its strictly indispensable nature for the dwarf sunflower and for barley, and thereby confirm the findings of MAZÉ with regard to the indispensable nature of zinc for maize and his general conclusion respecting its need by higher green plants.

Discussion

The foregoing discussion, and the photographic evidence furnished herewith, together with the evidence and hypotheses of MAZÉ, and the evidence of McHARGUE, WARINGTON, and SOMMER, which is cited above, render it clear that the concepts of present-day plant physiology with respect to the identity of all the chemical elements indispensable for higher green plants require fundamental revision. We can no longer maintain the list of ten essential chemical elements as one containing all of the kinds of elementary raw materials which are required for the making of cell tissues or for the metabolic processes of cells. Our studies furnish a strong presumption in favor of the view that the list of essential elements will probably be much lengthened when the subject is fully investigated, besides proving conclusively that boron and zinc must now be added to the list. Moreover, they indicate clearly that the ordinary technique of culture solution experiments is wholly inadequate for the solution of the subtler phases of the general problem. Nowhere in plant physiological investigations is a more painstaking and delicate control of experiments necessary, but with such methods employed, many new and important facts may be discovered.

In spite of the strikingly clear-cut evidence which we furnish herewith regarding the indispensable character of boron and zinc for green plants, the most difficult feature of the problem still remains unsolved. We have not even a good clue relative to the manner in which these elements function in cell metabolism. In that regard, however, we are not much more in the dark than we are with respect to the corresponding problem for the other essential elements. This is particularly true for elements like potassium and possibly calcium, organic compounds of which have never been convincingly proved to exist in plants. But it is also true of other essential elements which enter into the structure of organic molecules characteristic of plants; because it is not likely that some of them, at least, do not function in other vital ways than in that of contributing to the composition of characteristic organic compounds of plants.

To speak of the catalytic effect of an element like zinc merely expresses in other language our ignorance in respect to its actual function. This is a subject of such vital importance that no time should be lost in attacking it. Moreover, in the present state of our knowledge, definite methods of

experimental attack on that problem readily suggest themselves. It would be very surprising to us if experiments carried out *in vitro* under certain conditions would not yield important clues and possibly even solutions of that fundamental aspect of the problem of the essential chemical elements.

Be that as it may, one can no longer subscribe, in view of our evidence and that of other plant physiologists cited above, to the view that chemical elements required only in very small quantity by plants are to be regarded not as essential but merely as stimulating elements. A composite of the views of this category held generally in plant physiological circles is furnished in KOSTYTSCHÉW's *Lehrbuch der Pflanzenphysiologie*, which bears the date of the current calendar year. We submit that in view of the foregoing evidence and discussion, that position is untenable. It is also worthy of note that no text or reference book on plant physiology available shows that the author has apprehended the subject of this paper in any satisfactorily critical way. This seems inexcusable in the light of the investigations of those whose work we have cited above.

Conclusions

1. Photographic evidence and discussion are furnished in this paper which prove conclusively that boron and zinc are indispensable to the life and growth of a considerable number of widely different, higher green plants.

2. The experimental technique necessary to the solution of that problem is given in detail and shown to be the crux of the problem.

3. A general discussion is given of the few modern contributions on the general subject of essential chemical elements for plants in the light of a critical examination of earlier views and experiments.

The experiments reported above constitute a part of a more extensive investigation on the essential chemical elements for green plants. This investigation is made possible through a generous grant from the research funds of the UNIVERSITY OF CALIFORNIA to the junior author. Acknowledgment is gladly made to the President of the University and to the Board of Research for the assistance thus rendered.

THE UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA

LITERATURE CITED

1. BRENCHLEY, W. E., and THORNTON, H. G. The relation between the development, structure and functioning of the nodules on *Vicia faba*, as influenced by the presence or absence of boron in the nutrient medium. *Proc. Roy. Soc. London B* 98: 373-399. 1925.

2. MAZÉ, P. Influences respectives des éléments de la solution minérale sur le développement du maïs. *Ann. Inst. Pasteur* **28**: 21–68. 1914.
3. ————. Détermination des éléments minéraux rares nécessaires au développement du maïs. *Compt. Rendu Acad. Sci.* **160**: 211–214. 1915.
4. ————. Recherche d'une solution purement minérale capable d'assurer l'évolution complète du maïs cultivé à l'abri des microbes. *Ann. Inst. Pasteur* **33**: 139–173. 1919.
5. MCHARGUE, J. S. The rôle of manganese in plants. *Jour. Amer. Chem. Soc.* **44**: 1592–1598. 1922.
6. ————. Effect of different concentrations of manganese sulphate on the growth of plants in acid and neutral soils and the necessity of manganese as a plant nutrient. *Jour. Agr. Res.* **24**: 781–794. 1923.
7. ————. Manganese and plant growth. *Jour. Ind. Eng. Chem.* **18**: 172–175. 1926.
8. WARINGTON, KATHERINE. The effect of boric acid and borax on the broad bean and certain other plants. *Ann. Botany* **37**: 629–672. 1923.

SOME CHEMICAL CHANGES INCIDENT TO RIPENING AND STORAGE IN THE GRIMES APPLE*

FISK GERHARDT

(WITH SIX FIGURES)

Introduction

Since 1906, the Pomology Section of the Iowa Station has had under investigation a number of problems dealing with the storage of Iowa apples (1, 7, 10). The investigations have dealt principally with the development and control of Jonathan-spot, soft-scald and internal breakdown as these are affected by the maturity of fruit, cultural conditions, temperature, humidity, aeration and wrap experiments.

During the season of 1923, a joint project was outlined with the Chemistry Section of this Station which included a rather complete chemical study of Grimes apples handled under varying conditions before the storage period. It was particularly desirable to know more about the general chemical relationships existing within apples handled under known conditions.

The literature dealing with the chemical changes taking place in apples on the tree has been thoroughly reviewed by BIGELOW, GORE and HOWARD (2), also by MAGNESS and DIEHL (8). The present paper gives a rather complete account of certain chemical changes taking place in Grimes apples at the time they were placed in storage, after certain definite periods of delay, and at the time of picking. Further analyses of the fruit after removal from cold storage have been made and are included in this report. These data are of interest in connection with the storage experiments where certain physiological disorders have developed.

Collection of samples

Samples which were used in the following chemical investigations were part of the regular lots which were placed in cold storage. The plan adopted provided for the harvesting in a series of four pickings, three boxes each, storing immediately one box of each series at the time of picking and holding the other two boxes at the packing house for one and two weeks respectively before storing. At the time of each picking, a sample consisting of fifteen apples was selected from each storage lot for the chemical analysis. The treatment of these samples at the orchard was the same as

* A presentation of the chemical phase of a cooperative project between the Pomology and Chemistry Sections on the general problem of apple storage.

that of the corresponding storage lots. When a certain storage lot was sent by express to Ames for cold storing, a corresponding sample was sent to the Chemistry Section Laboratory for analysis. Each allotment of fruit reached Ames the morning following the day it was sent, the chemical samples representing the storage lots of fruit were shipped to Ames by Special Delivery, arriving at the laboratory within twenty-four hours.

Preparation of material for analysis

The apples arriving at the laboratory were immediately cut in thin slices, aliquot samples were taken for moisture determinations, and for expression of the juice by the aid of the hydraulic press. The remainder of the material was dried in a vacuum oven at 70° C. and 50 mm. pressure. This drying method permitted a minimum of metabolic changes in the tissue without causing caramelization of the carbohydrates. The dried material was ground in an "Empire" and "Merker" mill respectively until the ground product could be passed through a 100 mesh sieve.

Methods of analysis

A weighed sample of the ground tissue was freed from lipoids and soluble organic acids by percolation with anhydrous, alcohol-free ether. After expulsion of the ether the residue of the sample was extracted with boiling 90 per cent. alcohol (to which a small amount of CaCO_3 was added) for one-half hour on a hot plate. The filtered extract was concentrated to about 15 cc. at 50° C. and 20 mm. pressure. It was then diluted with water, clarified with neutral lead acetate, freed from excess lead by sodium carbonate, and finally made up to definite volume.

Reducing sugars.—Reducing sugars were determined by the MUNSON-WALKER method (9).

Total sugars.—Aliquot portions from the original sugar extracts were hydrolyzed with 2.5 per cent. HCl in the usual manner. Total sugars were determined by the DEFREN-O'SULLIVAN method (6).

Dextrins.—The residue from the sugar extraction was further extracted with 10 per cent. alcohol at a temperature of 50° C. for one-half hour. The filtered extract was concentrated for removal of alcohol and clarified by the above method. After hydrolysis with 2.5 per cent. HCl for 2.5 hours, the neutralized solution was made up to volume. Using the DEFREN-O'SULLIVAN method, the glucose value obtained was computed to the equivalent value for dextrin.

STARCH.—The residue from the dextrin extraction was boiled with 150 cc. of water for one minute. After cooling at 38° C. and digesting with fresh saliva until a negative result was obtained with iodine, the filtered solution

was hydrolyzed with 2.5 per cent. HCl for 2.5 hours. Glucose was determined by the DEFREN-O'SULLIVAN method, multiplied by the starch equivalent, and expressed as percentage of starch.

Hemicellulose or acid hydrolyzable.—The residue from the starch digestion was boiled with 150 cc. of 2.5 per cent. H_2SO_4 (by weight) for one hour. The filtered solution was neutralized and clarified in the usual manner. The acid-hydrolyzable portion was expressed in terms of glucose, using the DEFREN-O'SULLIVAN method.

Soluble pectin.—Five grams of the dry ground apple tissue were successively extracted with cold water at room temperature. It was found that three extractions removed all but traces of soluble pectin. The combined extracts were neutralized and brought to volume. Aliquots were taken for soluble pectin determination by precipitation as calcium pectate according to the method of CARRÉ and HAYNES (5).

Insoluble pectin.—The residue from the soluble pectin extraction was autoclaved with N/20 HCl for one hour according to Carré (4) for insoluble pectin determination.

Experimental

It was thought advisable to include a brief study of the changes occurring in the fruit prior to commercial picking. Accordingly samples of rather unripe apples were gathered and analyzed at stated intervals. These data together with the analyses of the various commercial pickings, both for immediate and for delayed storage are given in table I. These analyses were made to represent the composition of the apples before placement in cold storage.

Examination of these data will show that the ripening process in the apple is accompanied by decided changes in chemical composition. Collections prior to commercial picking show a higher moisture content, low specific gravity, low percentage of sugars, high dextrin and acid-hydrolyzable values, together with larger amounts of insoluble and total pectin, while the starch values during this period have not attained their maximum. During the process of ripening upon the tree, as shown by the various commercial pickings, the fruit remains quite constant in moisture content. Specific gravity and total sugars increase. Acidity decreases slightly with an accompanying decrease in starch and acid-hydrolyzable material. The various forms of pectin remain quite constant during the commercial picking stage, substantiating the statement of CARRÉ (4) "that the maximum pectin content continues over a period of about four weeks, during which the apples remain in their prime condition."

Apples delayed one week before storage, continue their ripening processes as shown by a decrease in moisture, acidity, dextrins, starch and acid-

TABLE I
COMPOSITION OF GRIMES APPLES DURING VARIOUS STAGES OF MATURITY

DATE OF PICKING	DATE OF ANALYSIS	MOISTURE PER CENT.	SPECIFIC GRAVITY	ACIDITY CC. N/10 10 CC. JUICE	REDUCING SUGARS PER CENT.	TOTAL SUGARS PER CENT.	DEXTRINS PER CENT.	STARCH PER CENT.	ACID HYDROLYZABLE PER CENT.	SOLUBLE PECTIN PER CENT.	INSOL. PECTIN PER CENT.	TOTAL PECTIN PER CENT.
Collected prior to commercial picking												
Aug. 14	Aug. 15	87.00	1.0300	9.40	23.03	23.20	2.17	13.24	11.48	0.62	3.43	4.06
Aug. 29	Aug. 30	86.76	1.0355	8.25	25.24	30.95	2.05	10.50	7.28	0.72	3.00	3.70
Sept. 3	Sept. 4	85.00	1.0385	9.60	25.08	35.36	2.05	15.98	8.25	0.63	3.14	3.77
Average		86.21	1.0343	8.98	24.44	29.83	2.09	13.26	9.00	0.64	3.19	3.84
Commercial pickings—immediate storage												
Sept. 14	Sept. 15	85.36	1.0410	10.00	27.44	33.08	0.93	19.86	4.92	0.66	2.72	3.38
Sept. 21	Sept. 22	85.12	1.0440	9.10	24.00	63.04	1.35	17.28	3.16	0.65	2.92	3.57
Sept. 25	Sept. 26	85.90	1.0465	9.60	24.72	65.29	3.79	13.86	3.16	0.63	2.60	3.23
Oct. 1	Oct. 2	87.53	1.0465	8.40	26.88	45.92	0.00	18.07	3.87	0.46	2.64	3.10
Average		85.98	1.0445	9.27	25.76	51.83	1.51	17.26	3.77	0.60	2.72	3.32
Storage delayed 1 week												
Sept. 14	Sept. 22	84.43	1.0420	8.90	28.21	78.24	3.63	15.98	3.33	0.63	2.98	3.63
Sept. 21	Sept. 28	87.00	1.0495	9.20	26.16	86.72	1.10	10.33	2.99	0.76	3.04	3.80
Sept. 25	Oct. 3	85.71	1.0515	7.50	27.50	48.64	0.00	8.28	1.50	0.84	2.32	3.16
Oct. 1	Oct. 9	85.04	1.0505	8.40	25.76	55.04	0.00	10.15	1.76	0.66	2.78	3.44
Average		85.54	1.0483	8.50	26.90	67.16	1.18	11.18	2.39	0.72	2.78	3.50
Storage delayed 2 weeks												
Sept. 14	Oct. 1	85.00	1.0535	8.00	29.68	58.08	0.00	6.19	1.60	1.14	2.40	3.54
Sept. 21	Oct. 6	86.32	1.0545	8.40	28.64	38.08	0.00	3.61	2.46	1.24	2.30	3.54
Sept. 25	Oct. 11	84.65	1.0588	8.00	30.00	55.84	0.00	5.28	1.58	1.20	2.48	3.68
Oct. 1	Oct. 16	83.81	1.0582	7.80	29.36	59.58	0.00	4.42	2.28	1.00	2.64	3.64
Average		84.94	1.0562	8.05	29.42	57.90	0.00	4.87	1.98	1.14	2.45	3.60

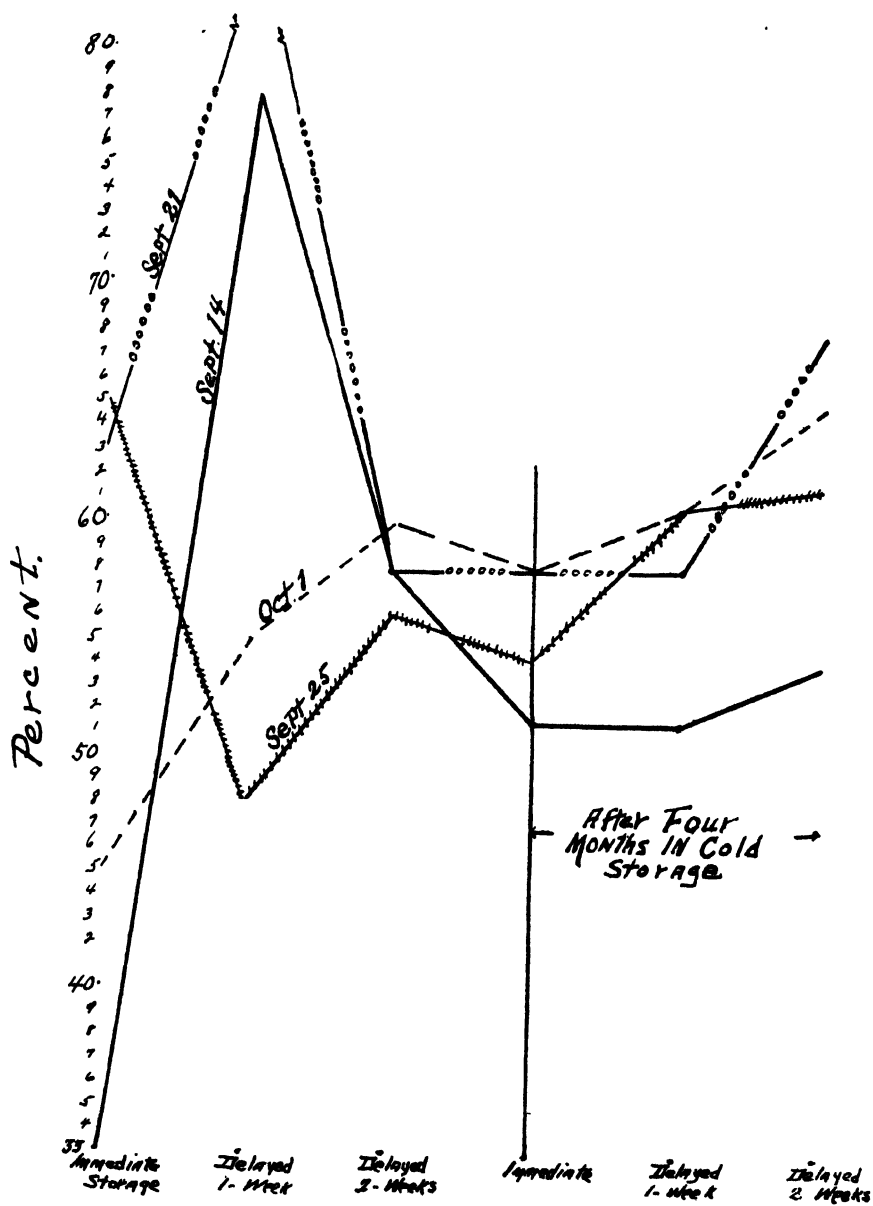


FIG. 1. The effect of different picking dates and storage conditions upon total sugars.

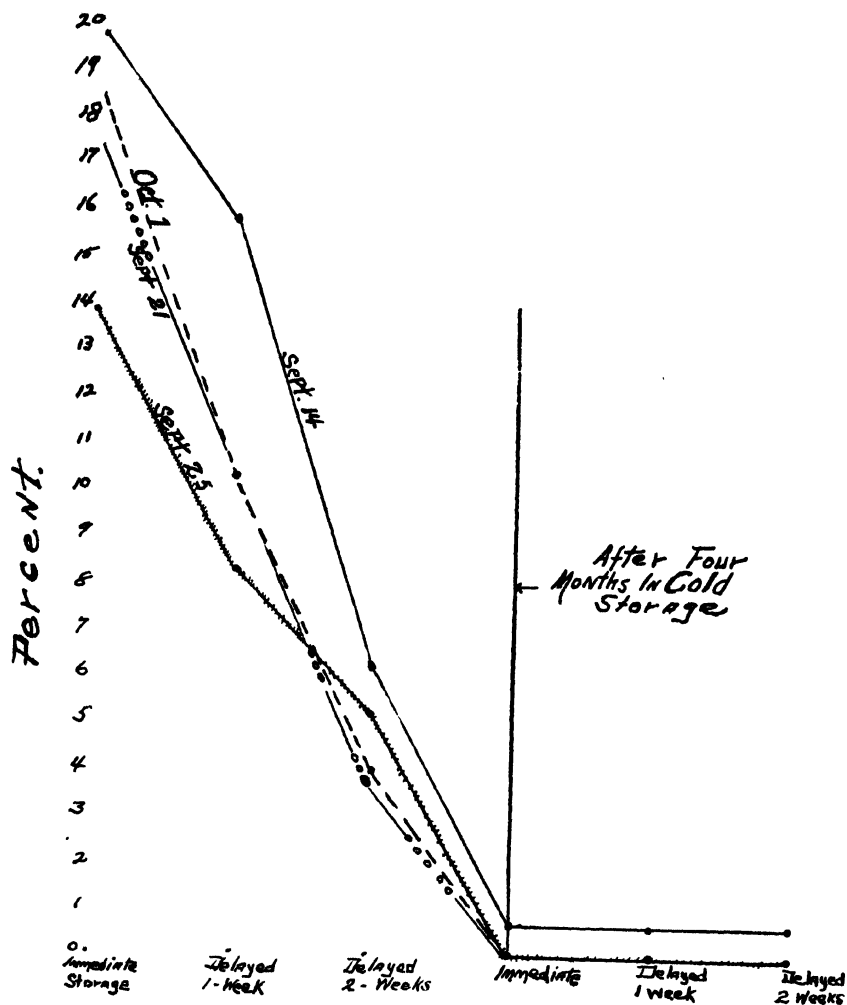


FIG. 2. Starch values at different pickings and storage conditions.

hydrolyzable material, with a corresponding increase in specific gravity, sugars and soluble pectin. It is interesting to note the decided increase in disaccharides produced by delayed storage of one week. This increase in disaccharides produced by one week delayed storage is shown in fig. 1, the first commercial picking of September 14th showing an increase of 73 per cent. total sugars. During this period the starch values have decreased only about 20 per cent. as shown in fig. 2; while simple reducing sugars in fig. 3 remain quite constant. Apples delayed two weeks prior to storage

show a continuance of the ripening processes. During this period the average percentage of total sugars decline markedly, suggesting the probability of rapid respiration. Soluble pectin shows a decided increase at the end of two weeks delayed storage, indicating a stage of complete maturation. Since the values for simple reducing sugars remain quite constant throughout the entire series, a portion of the increase in disaccharides might be accounted for by direct elaboration through hydrolysis of starch and acid-hydrolyzable matter. Browne in his study of the chemistry of the apple

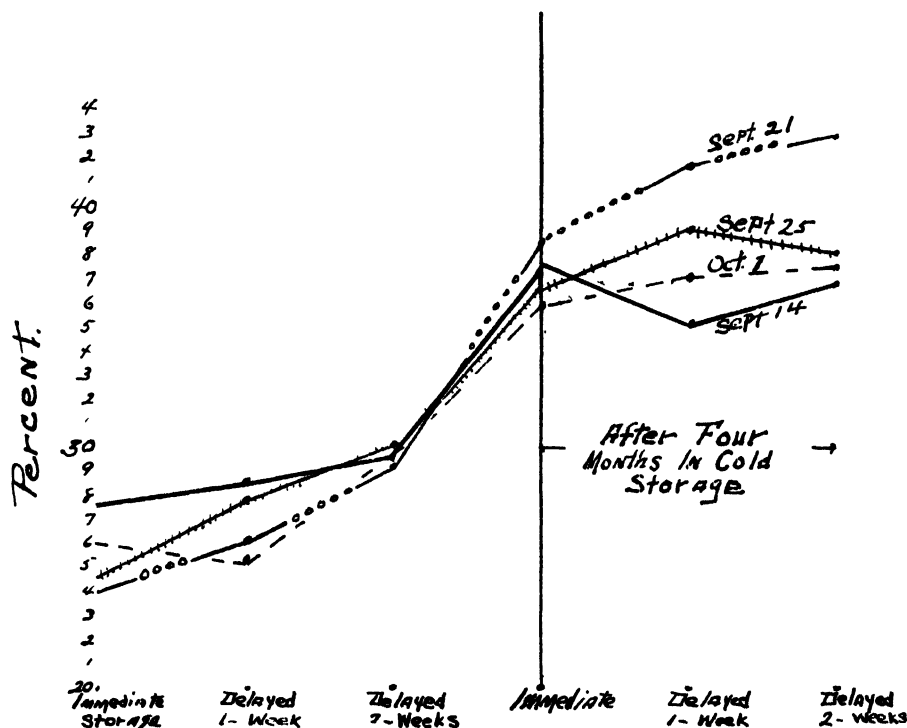


FIG. 3 Reducing sugar values at various picking dates and storage conditions.

(Bull, 58, Penn. Dept. of Agr.) states: "With increase of sugar we notice a marked decrease in the percentage of starch—a coincidence which leads to the supposition that starch may serve as a parent substance in the formation of sugar in apples, and that sucrose is the first product produced." Examination of figs. 1, 2, 3, and 4 shows that after two weeks of delayed storage, starch, acidity, total and reducing sugars tend to approximate more uniform values irrespective of date of picking or stage of maturity. The values for pectin indicate a general trend, rather than a definite correlation,

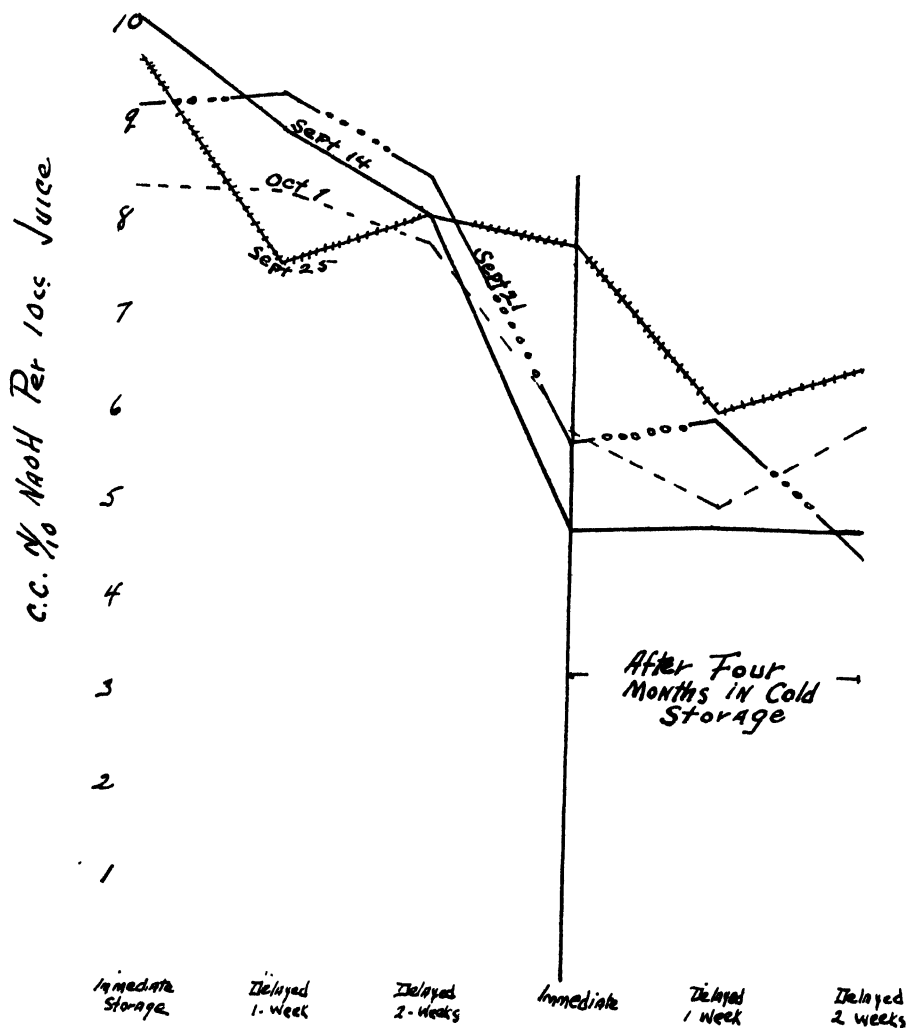


FIG. 4. Acidity changes at various pickings and storage treatments.

due to fluctuation in values probably accounted for by the variable state of maturity of the individual apples sampled.

Cold storage analysis

After remaining in cold storage for a period of four months, representative samples corresponding to those in table I were again analyzed. Notations were made upon each lot as to keeping qualities. The percentage of sound apples, free from scald and breakdown, was determined in each crate.

TABLE II

COMPOSITION OF COLD STORED APPLES, JANUARY, 1924

DATE OF PICKING	SPECIFIC GRAVITY	ACIDITY CC. N/10 NaOH	REDUCING SUGARS PER CENT.	TOTAL SUGARS PER CENT.	DEXTRIN PER CENT.	STARCH PER CENT.	ACID HYDROLYZABLE PER CENT.	SOLUBLE PECTIN PER CENT.	INSOLUBLE PECTIN PER CENT.	TOTAL PECTIN PER CENT.	APPLES FREE FROM BREAK-DOWN PERCENTAGE	APPLT-SOALD PERCENTAGE
							Immediate storage					
Sept. 14	1.0505	4.8	37.52	51.52	0.63	0.63	3.20	1.28	3.28	4.56	99.6	60.0
Sept. 21	1.0600	5.6	38.56	58.08	0.40	0.00	2.84	0.80	2.40	3.30	92.5	53.3
Sept. 25	1.0635	7.8	37.52	53.60	0.63	0.63	1.41	1.28	2.88	4.16	55.3	20.0
Oct. 1	1.0540	5.7	36.40	58.88	0.23	0.00	2.46	0.40	2.64	3.04	95.5	0.0
Average	1.0570	5.95	37.50	55.42	0.47	0.31	2.47	0.94	2.80	3.76	85.7	33.3
							Storage delayed 1 week					
Sept. 14	1.0475	4.8	35.04	51.30	0.63	0.63	2.31	0.96	3.12	4.08	81.5	86.8
Sept. 21	1.0635	5.8	41.84	58.12	0.35	0.00	1.40	0.64	2.48	3.12	89.9	33.3
Sept. 25	1.0540	6.0	39.28	60.32	0.50	0.00	2.31	0.68	3.20	3.88	100.0	13.3
Oct. 1	1.0475	5.2	37.12	60.50	0.28	0.00	2.46	0.50	2.70	3.20	63.3	0.0
Average	1.0530	5.45	38.32	57.56	0.44	0.15	2.12	0.69	2.87	3.57	83.42	33.4
							Storage delayed 2 weeks					
Sept. 14	1.0580	4.5	36.80	54.40	0.27	0.63	1.95	1.28	2.64	3.92	89.1	93.3
Sept. 21	1.0510	4.5	42.56	68.00	0.40	0.00	1.97	1.00	2.64	3.64	99.5	33.3
Sept. 25	1.0560	6.5	38.58	60.30	0.34	0.00	2.14	0.52	2.96	3.48	99.3	40.0
Oct. 1	1.0565	5.8	37.52	65.92	0.34	0.00	2.32	0.44	2.56	3.00	90.0	0.0
Average	1.0553	5.32	38.86	62.15	0.38	0.15	2.09	0.81	2.70	3.51	94.47	41.65

Only sound apples were used for the chemical determinations. The results found in table II give a picture of the changes in carbohydrate content occurring during cold storage.

Inspection of these data shows the uniformity of all cold storage material irrespective of previous differences in stage of maturity. In other words the time of picking, within certain limits, influences but little the ultimate chemical composition of apples passing through cold storage. The ripening processes previously noted continue much more slowly under cold storage conditions. Acidity, dextrins, starches, and acid hydrolyzable material

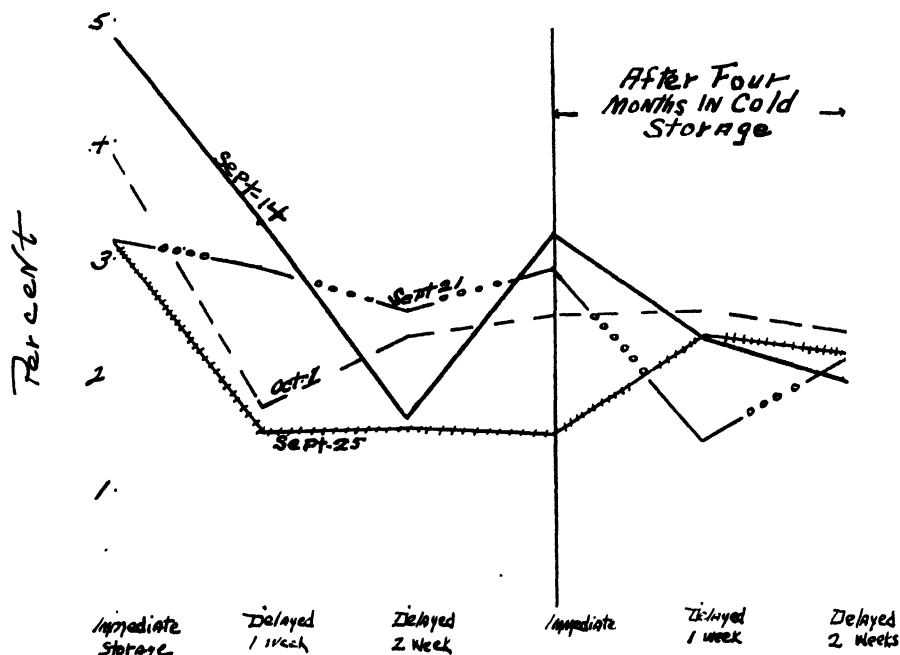


FIG. 5. Percentage hemicellulose at various pickings and storage treatments.

gradually decrease, with a corresponding slight increase in simple and disaccharide sugars. The pectin values show little or no response to pre-storage treatment. In fig. 6 apple scald appears to be correlated with maturity of fruit or time of picking, as previously shown by PLAGGE (10). The curves show a progressive decrease in scald tissue with increase in maturity. However, the chemical constants shown in figs. 1, 2, 3, 4, and 5 show little or no relation to the production of apple scald.

The percentage of breakdown in fig. 6 increases with late picking as with delayed storage, late picking with immediate storage yielding approximately the same amount of breakdown as early picking with late

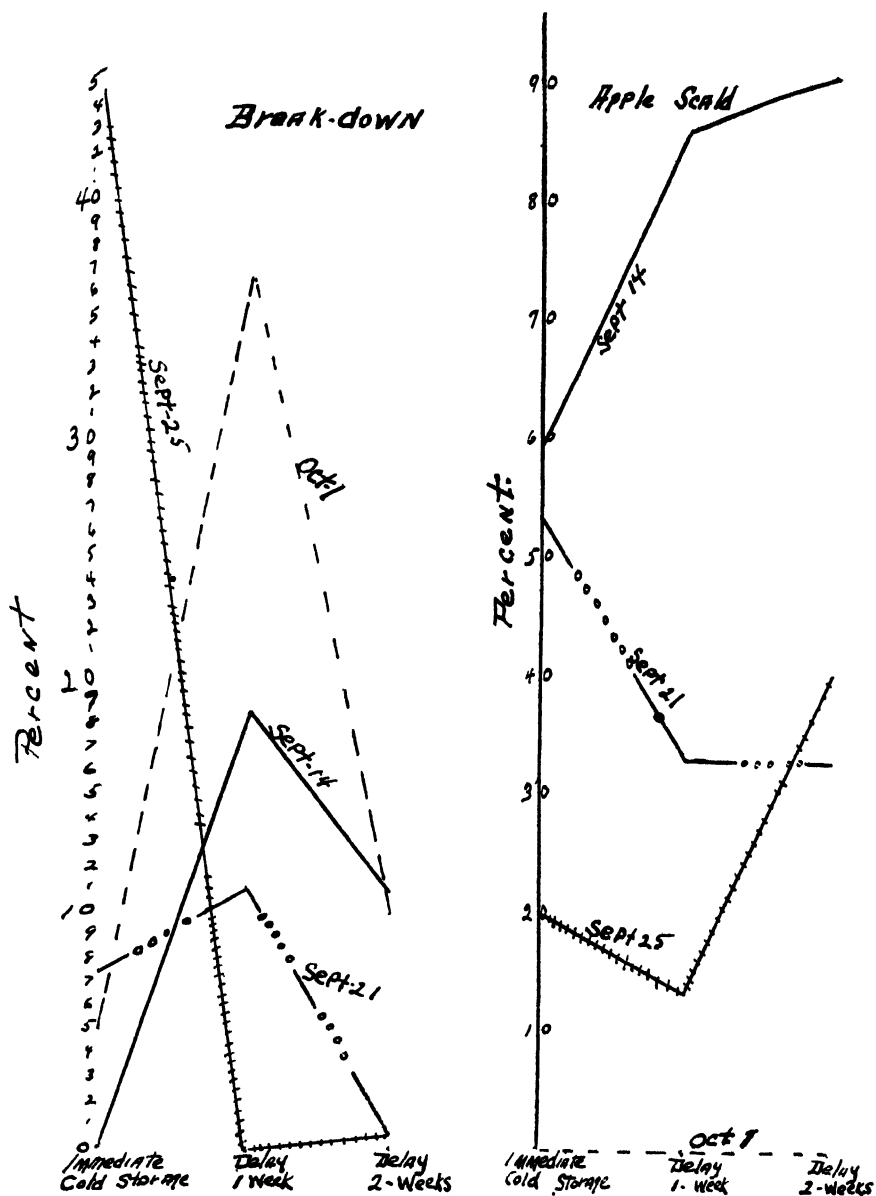


FIG. 6. Percentage of breakdown and apple scald at various pickings and storings, during the cold storage period.

TABLE III
COMPOSITION OF NORMAL AND BREAKDOWN COLD STORAGE APPLE TISSUE

Lot No.		SPECIFIC GRAVITY	ACIDITY CC. N/10 NaOH PER 10 CC. JUICE	PERCENTAGE REDUCING SUGARS	PERCENTAGE TOTAL SUGARS	PERCENTAGE DEXTRIN	PERCENTAGE STARCH	PERCENTAGE ACID HYDROLYZABLE	PERCENTAGE SOLUBLE PECTIN	PERCENTAGE INSOL. PECTIN	PERCENTAGE TOTAL PECTIN
1	Normal tissue	1.0635	7.8	37.52	53.60	0.63	0.63	1.41	1.28	2.88	4.16
	Breakdown tissue	1.0580	5.2	40.00	56.80	1.70	0.00	2.40	0.40	3.20	3.60
2	Normal tissue	1.0635	5.8	41.84	58.12	0.36	0.00	1.40	0.64	2.48	3.12
	Breakdown tissue	1.0530	4.4	47.60	56.95	1.75	0.00	2.10	0.40	3.20	3.60

storage. A comparison of breakdown curves in fig. 6 with those for total sugars, prior to cold storage, in fig. 1 suggests a certain amount of interrelationship. The minimum and maximum points in these figures assume somewhat like appearances. Considering the various pickings individually, it will be seen that low total sugars are followed by low breakdown. With an increase in total sugars, as shown by one week's delayed storage there is also an increase in breakdown. Vice versa a decrease in total sugars of the individual pickings shows a decrease in breakdown. The September 25th picking has a negative total sugar curve and also a negative breakdown curve. On the basis of individual pickings it appears that low total sugars are associated with low breakdown. However, when averaging the various pickings and using definite values the above relationship does not hold. For example the pickings on September 14th and 21st have the highest percentage of total sugars and the lowest percentage of breakdown. It would appear then if there is an interrelationship between total sugars and breakdown, that it is a function of individual pickings and not a finite value. With the exception of the relation of total sugars to breakdown there appear to be no definite correlations between chemical composition as determined, and keeping qualities in the sample of Grimes apples used in this work.

According to SANDO (11), certain unfavorable changes occurring during apple storage are associated with the presence of a chromogenic substance, quercetin or its glucoside. While BROOKS, COOLEY and FISHER (3) state that "their experiments indicate that apple-scald is due to an accumulation of esters or similar products in the tissues of the fruit."

Since both apple-scald and breakdown were found in the cold storage material, a chemical analysis was made upon normal and diseased tissue in several crates. In each sample the soft, dark brown tissue was removed from the sound portion of the apple. These tissues were dried separately in the vacuum oven at 70° C. and 50 mm. pressure. The results of these analyses appear in table III.

The unsound tissue shows a lower specific gravity, acidity, and soluble pectin content, together with a higher amount of acid hydrolyzable material. However, on the basis of these few analyses the differences in chemical composition apparently do not account for the extreme variation in texture and appearance of the tissues.

Summary

1. A chemical study has been made of the changes incident to ripening and storage in the Grimes apple.
2. The ripening process both on the tree and in storage is associated with loss of moisture, acidity, dextrins, starch, and acid hydrolyzable material, together with an increase in specific gravity, sugars and soluble pectin.

3. The time of picking or condition of maturity, within certain limits, affects but little the ultimate chemical composition of the cold storage apples in this work.

4. The production of apple scald tissue tends to decrease with increase in maturity of the fruit.

5. A certain interrelationship exists between the relative amount of total sugars in each individual picking of the fruit prior to cold storage and production of breakdown tissue.

6. With the exception of total sugars there appears to be little, if any, correlation in this study between chemical composition and keeping quality of cold storage apples.

7. Chemical differences exist between normal tissues and those showing internal breakdown. These differences, however, are relatively small and suggest that physical or other chemical changes not determined are involved in the formation of unfavorable tissue during storage.

IOWA AGRICULTURAL EXPERIMENT STATION,
AMES, IOWA

LITERATURE CITED

1. BEACH, S. A., and EUSTACE, H. J. Cold storage for Iowa-grown apples. Iowa Expt. Sta. Bull. **108**. 1909.
2. BIGELOW, W. D., GORE, H. C., and HOWARD, B. J. Studies on apples. U. S. Dept. Agr. Bur. Chem. Bull. **94**. 1905.
3. BROOKS, CHARLES, COOLEY, J. S., and FISHER, D. F. Nature and control of apple scald. Jour. Agr. Res. **18**: 211-240. 1919.
4. CARRÉ, MARJORIE H. An Investigation of the changes which occur in the pectic constituents of stored fruit. Biochem. Jour. **16**: 704-712. 1922.
5. CARRÉ, MARJORIE H., and HAYNES, DOROTHY. The estimation of pectin as calcium pectate and the application of this method to the determination of the soluble pectin in apples. Biochem. Jour. **16**: 60-69. 1922.
6. DEFREN, GEORGE. The determination of reducing sugars in terms of cupric oxide. Jour. Amer. Chem. Soc. **18**: 749-766. 1896.
7. GREEN, LAURENZ. Cold storage for Iowa-grown apples. Iowa Expt. Sta. Bull. **144**. 1913.
8. MAGNESS, J. R., and DIEHL, H. C. Physiological studies on apples in storage. Jour. Agr. Res. **27**: 1-38. 1924. Literature cited pp. 36-38.
9. MUNSON, L. S., and WALKER, PERCY H. Unification of reducing sugar methods. Jour. Amer. Chem. Soc. **28**: 663-686. 1906.
10. FLAGGE, H. H., and MANEY, T. J. Apple storage investigations. Iowa Expt. Sta. Bull. **222**. 1924.
11. SANDO, CHARLES E. The isolation and identification of quercetin from apple peels. Jour. Agr. Res. **28**: 1243-1245. 1924.

GROWTH STUDIES ON FRUITS*

FELIX G. GUSTAFSON

(WITH FOUR FIGURES)

In recent years the literature has contained numerous investigations on the rate of growth. These have ranged from studies on the rate of growth of the dairy cow to bacteria. Increase in length, breadth, and weight have all been used as criteria of growth. Among these investigations there is no mention of the growth of fruits. In fact, as far as the writer is aware, only one single experiment has ever been published in which the growth of a fruit was measured throughout the growth period.¹ This was an experiment by ANDERSON (1) in 1895 on the fruit of *Cucurbita Pepo*, in which weight was measured by a self registering balance. The growth of fruits is somewhat different from that of other plant organs, being mainly a storing up process, and for this reason it has been thought worth while to investigate their growth, also.

In the summer of 1924 preliminary studies of the growth of fruits were commenced. The intention in the beginning was to measure growth of individual fruits by weighing them on the plant at intervals of a week during the growing season. To do this it would be necessary to select easily grown fruits of considerable size, with long and flexible peduncles. The plants finally selected were: *Cucurbita Pepo* var. *condensa* (scalloped summer squash), *Cucumis Melo* (muskmelon), *C. sativus* (cucumber), and *Lycopersicon esculentum* (tomato).

It soon became obvious that it would not be feasible to measure growth by the increase in weight of fruits on the vines. Therefore another method was developed, namely the measurement of the volume of a fruit by the displacement of water in a calibrated container. By this method the increase in growth, as represented by increase in volume, could be measured fairly rapidly and accurately with the least disturbance of the plant and without injuring the fruit. The first measurement was made at the time when the blossoms had just appeared. At this time the young fruits (ovaries) differed somewhat in size, but physiologically they were of the same age. In the case of the tomato it was necessary to obtain an average volume of the ovary by collecting a large number (200 to 400) from flowers

* Papers from the Department of Botany of the University of Michigan, No. 248.

¹ Since writing this Professor H. S. REED has informed the writer that J. GALINSKA has published a paper on growth of fruits. This paper, however, has not been available.

of the same age and then measuring them all together and thus obtaining an average. The plants were grown out of doors with the exception of the cucumbers and summer squashes, which were grown in twelve inch pots in an open green house during the warm part of the summer and later in a heated green house. These plants were started too late to mature out of doors.

Between July 28 and August 4 forty-four muskmelon blossoms were tagged and the ovaries measured. At this time the ovary volume ranged from 0.5 to 2.5 cc. Though the fruits were carefully handled, the majority of the tagged fruits never developed to any size, living for a few weeks with-

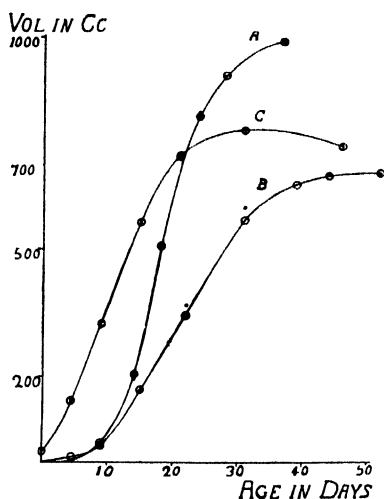


FIG. 1. Growth curves. Ordinates represent the volume of the fruits in cubic centimeters, abscissa age in days. Curve A, average of seven mature fruits of muskmelon; B, average of six cucumbers; C, average of three scalloped summer squashes. The dots on curves A and B, theoretical values calculated according to the equation

$$\log \frac{x}{A-x} = K(t-t_1).$$

out gain in size and then withering. Of the forty-four originally labeled only twenty developed to any size and only seven survived until entirely ripe. Most of the other twenty were destroyed by rodents, when nearly ripe. Curve A, fig. 1, gives the general character of the growth curves obtained when size in cubic centimeters was plotted against age in days. This curve is an average of the seven fruits that matured. The final size ranged from 700 to 1,200 cc. Since each separate growth curve had the same general shape as the average it seems permissible to use the average of them rather than to choose one that was representative.

As stated before, eighteen cucumber plants were grown in the green house. As the blossoms opened fifty-five flowers were tagged and the ovaries measured. Most of these developed, but only a few matured, for the plants were unable to support more than two maturing fruits and usually only one. In these fruits, as in the muskmelons, there would sometimes be a lag period of several weeks, from the time of blossoming till the fruit began rapid growth. From the twelve that fully matured, six that were most nearly the same size have been used to construct curve B, fig. 1. Growth curves have been constructed from all fruits that matured and they all show the same general characteristics, a slow increase in the beginning, followed by a very rapid increase, which in turn ends by a gradual decrease and a final cessation of growth.

Twelve plants of scalloped summer squash were grown under similar conditions. Fruits did not set very freely and only three matured. For the sake of completeness they are included. In these fruits, that part of the growth which is very slow precedes the blossoming; and when measurement began the ovaries were already of some size. On blossoming they immediately commenced to grow very rapidly. This gives a steep curve unlike that usually obtained. In a few cases the ovaries were measured some time before the opening of the blossoms and these showed the characteristic slow growth during the early part of the development. Therefore if curve C, fig. 1, which represents the growth of scalloped summer squashes, were extended to the left it would resemble the usual type.

The measurements on the tomatoes were for the most part started too late for the fruits to mature. A few matured and here also the growth curve is like that of the others. As tomatoes were also used in the work of 1925 the curves of the few fruits which matured in 1924 are not included in fig. 1.

The four fruits studied in a preliminary way during the summer of 1924 all showed that growth proceeds in fruits in very much the same manner that it does in other plant parts and in animals. The number of individual fruits measured was rather small and it would not be safe to draw any far reaching conclusions from these measurements. For this reason it was felt that it might be worth while to continue the study in more detail. In the summer of 1925 two varieties of tomato were chosen, "John Baer" and "Livingston." These fruits were uniform in size, round and smooth, facilitating handling. The purpose this time was to obtain the green and dry weight as well as the volume. The dry weight was included because it might be objected that volume increase represented perhaps only an accumulation of water and that the accumulation of protoplasm, cell wall material and stored foods, which might be considered as representing growth, did not follow the increase in volume. To obtain the dry weight it was necessary to pick the fruits.

Somewhat over 200 plants were grown of each variety and on these plants several thousand blossoms were tagged between July 5 and August 13. From these plants fruits were collected at such times as to give fruits differing from one another in age by one week. Fifty fruits were collected of each age except in the case of the ovaries and the first two weeks after blossoming, when 200 and 75 respectively were collected.

Nine different collections were made from "John Baer," the ovaries at the time of blossoming, and one for each week afterward. The fruits ripened in eight weeks. It was found that fruits four or more weeks old would vary considerably in size. It was found that this variation depended upon the position of the fruit in a fruiting cluster. The one first to set was always the largest, and the succeeding fruits showed a gradual decrease in size. The last few blossoms did not set at all. To eliminate any error from this source fruits were as a rule collected from the same position in a cluster, and in the older collections, where the error would be greater, fruits of the same date of tagging were collected in successive weeks. Thus "John Baer" seven and eight weeks were both from blossoms tagged July 18. Another possible source of error was also discovered early enough to avoid it; the earliest fruits and the last fruits set on a plant are smaller than those set in between, irrespective of position in a fruiting cluster. For this reason only fruits tagged during the height of the vigor of the plant were used; those tagged between July 5 and 18 were not used, nor those tagged after August 11, as their fruits on maturing were much smaller than those tagged in between. The ideal condition would be to grow several thousand plants, tag on the same day identical blossoms and make collections only from these week after week. The writer, however, feels that he has overcome these sources of errors in all cases. The smoothness of the curves bears this out.

As soon as the fruits had been collected they were brought into the laboratory and the volume and green weight determined. The fruits were bisected by cutting along the main axis to facilitate drying, but after the third week (*i.e.*, after the fourth collection) only one half of each individual fruit was used for drying. These fifty half fruits were again weighed and partially dried in a drying oven constructed for drying mushrooms. The drying was completed in an electric oven at a temperature between 90 and 98° centigrade. From these dried halves the total dry weight of the fifty fruits was calculated.

At the same time that the fruits were collected and their volumes and weight determined, the volumes of others were measured directly on the plants as in the preceding summer. Fifty blossoms of "John Baer" were tagged July 5-15 and their volumes measured weekly. Of these fifty fruits thirty-nine matured and their volumes have been averaged.

Fig. 2 gives the volume measurements. Curve A represents the growth progress of the thirty-nine fruits measured on the vines of "John Baer" variety between July 5 and August 31. The plants were young at the time the fruits were selected, in fact many fruits were the first ones to set on the plants. Curve B represents the increase in volume of "John Baer" as obtained by picking the fruits at intervals of one week, from those tagged between July 18 and August 7. As already pointed out the first fruits on a vine are smaller than those set later when the plant is growing more vigor-

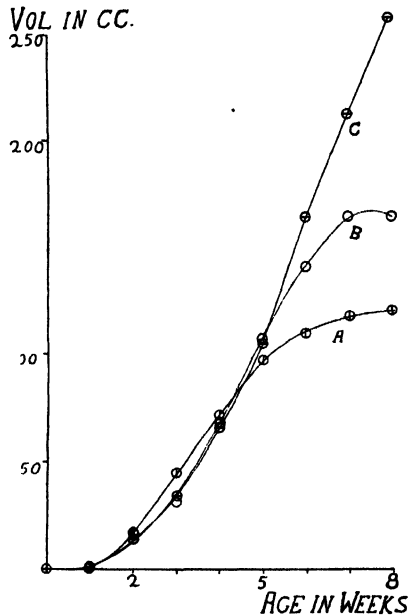


FIG. 2. Growth of tomatoes, measured by the increase in volume. Curve A, average of thirty-nine fruits of "John Baer" measured on the vine; B, average of fifty fruits of "John Baer" measured by picking the fruits at intervals of one week; C, average of fifty fruits of "Livingston" measured by picking the fruits at intervals of one week. The "Livingston" fruits were not quite ripe.

ously. This is illustrated by A and B; the latter reaching a volume 40 per cent. greater than the former, which were set for the most part July 5, when the plants were just starting to bear. Though not entirely alike, yet both curves show that the growth is slow at first, increases rapidly and again slows down. Curve C illustrates the increase in volume of the "Livingston" tomato. This tomato requires at least nine weeks to mature instead of eight weeks as does the "John Baer." The mature fruits have been left out because no representative fruits were left by that time, September 21, as the

moist conditions had brought about decay of the fruits that were tagged late in July. Those tagged earlier would not have represented the true conditions. The curve would seem to indicate that this tomato keeps on growing fairly rapidly until mature.

Curve A and C, fig. 3, represent the green weight of "John Baer" and "Livingston" respectively. These are the same individual fruits as shown in fig. 2, B and C. Curves B and D represent the dry weight of fruits in A and B multiplied by twenty. The curves representing green and dry weight are similar to the volume measurement, so that it seems as if volume measurement was as good a criterion of growth as any other.

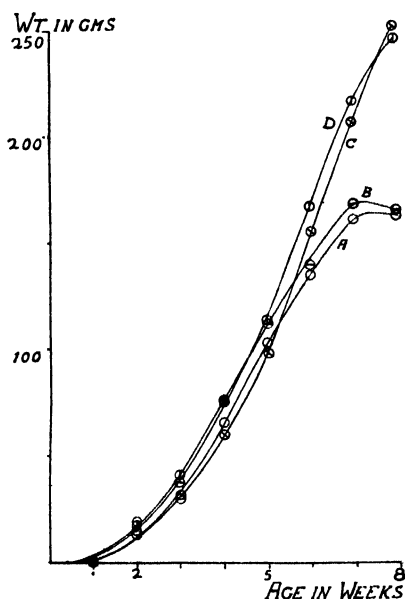


FIG. 3. Growth of tomatoes, measured by increase in green and dry weights, from the same fruits as in fig. 2, curves B and C. Curves A and C represent the green weight of "John Baer" and "Livingston" respectively, B and D the dry weights of A and C respectively, multiplied by twenty. The "Livingston" fruits were not quite ripe.

The percentage of dry weight is highest in the ovary, falling rapidly during the first two weeks and after that more slowly, but at no time is there an increase (fig. 4). The ovary of the "John Baer" variety contained 17 per cent. dry matter, when two weeks old the fruits contained only 6.2 per cent. and when mature, six weeks later, only 5.0 per cent. The "Livingston" contained 21 per cent. dry matter at blossom time, 6.3 per cent. two weeks later and when mature 4.6 per cent. MACDOUGAL (2) reports that young fruits contained 13 per cent. and mature 9 per cent. dry

matter. He dried the fruits in a beaker over a water bath at 100° and weighed them at the end of two days. It took the writer weeks to get constant weight when an electric oven was used. Therefore it seems that MacDougal's results for the mature fruit must be too high by several per cent.

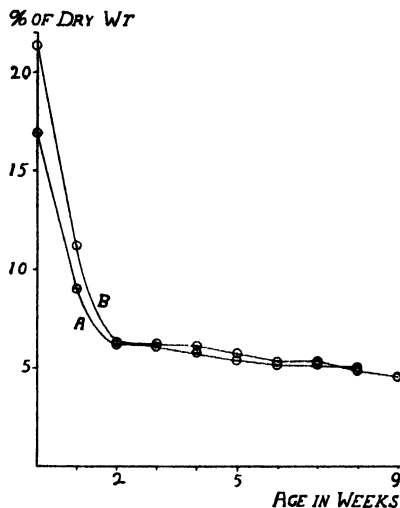


FIG. 4. Percentage of dry matter in the two varieties of tomatoes at different ages. Curve A, "John Baer"; B, "Livingston."

From the preceding data it seems as if the growth in fruits were very much like that found in animals and vegetative parts of plants. That is, at first there is a period of slow growth, gradually becoming more rapid until a point of maximum rate is reached, and from that time there is a slowing up. Plotting total volume or weight against time an "S" shaped curve is obtained.²

ROBERTSON (4) and OSTWALD (3) independently likened this to an autocatalytic reaction of the first order, which also gives an "S" shaped curve. Both of these writers, using their own as well as data of other investigators, compared the observed growth curves plotted as above with the calculated curves, using the equation $\log \frac{x}{A-x} = K(t-t_1)$, which is the equation representing a monomolecular autocatalytic reaction, where A represents final size reached, x size at time t , t_1 time at which half the final size is reached

² From time-rate curves constructed, it looks as if there were two growth cycles in the growth of the tomato fruit. These two cycles have appeared both when averages have been plotted and when individual records have been used. Thus eighteen individual growth records which were plotted all showed two maxima. This phase of the work is to be investigated further.

and K a constant. Both of these investigators obtained good agreements between the observed and calculated values, as have other investigators since. In fig. 1, curves A and B, the writer has compared the observed and calculated values in growth of muskmelon and cucumber, and the agreement is fairly good except in the early phase. In his recent book on growth ROBERTSON (5) has elaborated his theory much more extensively.

From the work here presented and from some not included it seems that Robertson's explanation of growth is not entirely satisfactory. However, controlled growth studies on fruits should be conducted; and from such experiments definite information may be gained, which would help in interpreting growth curves in other ways than as an autocatalytic reaction.

Summary

Growth measurements of fruits of *Cucurbita Pepo* var. *condensa*, *Cucumis Melo*, *C. sativus* and *Lycopersicum esculentum* are presented. Growth curves plotted for these show that the growth progress in these fruits is similar to that found for other plant parts and for animals.

THE UNIVERSITY OF MICHIGAN,
ANN ARBOR, MICHIGAN

LITERATURE CITED

1. ANDERSON, A. P. The grand period of growth in a fruit of *Cucurbita Pepo* determined by weight. Minnesota Bot. Studies 1: 238-279. 1894-1898.
2. MACDOUGAL, D. T. Hydration and growth. Carnegie Inst. Washington Publ. no. 297. 1920.
3. OSTWALD, WOLFGANG. Über die zeitlichen Eigenschaften der Entwicklungsvorgänge. (Roux's) Vorträge u. Aufsätze über Entwicklungsmechanik. Heft 5: 71 pp. 1908.
4. ROBERTSON, T. B. On the normal rate of growth of an individual, and its biochemical significance. Arch. f. Entwicklungsmech. d. Organismen 25: 580-614. 1907-08.
5. ————. The chemical basis of growth and senescence. Lippincott, Philadelphia. 1923.

PLANT GROWTH-PROMOTING SUBSTANCES, HYDROGEN ION CONCENTRATION AND THE REPRODUCTION OF *LEMNA**

NORMAN ASHWELL CLARK

(WITH ONE FIGURE)

Introduction

A considerable amount of investigation has been done during recent years upon the nutrition of green plants in connection with growth-promoting substances. Plants are able to produce vitamins without which animals cannot grow; and it has been claimed (1, 2, 9) that these green plants, in their turn, need an organic "auximone" or accessory substance in order to maintain their health and to reproduce. Evidence, however, is accumulating (4, 5, 7, 11, 12) that the auximone is not an essential factor, although it may have a stimulating action, and that green plants can grow in inorganic salt solutions if suitable conditions are given them. Among other conditions the hydrogen ion concentration of these solutions has been studied.

A large part of the work on the auximones has been done with different plants of the *Lemnaceae* or duckweed family. BOTTOMLEY (1, 2, 3) found that these could not grow satisfactorily in DETMER's or KNOP's solutions without organic matter being added. MOCKERIDGE (9, 10) checked BOTTOMLEY's results with the same two solutions, and found that there was a decided stimulating effect with various nucleic acid derivatives isolated from soil and manure. Both azotobacter and yeast contained growth-promoting material, and the suggestion was made that some of the auximones may be direct products of bacterial metabolism.

CLARK and ROLLER (6) reported that a solution of inorganic salts could be developed which was suitable for *Lemna major* (8) and in which good growth and reproduction could be obtained over an indefinite period without the addition of any organic matter. CLARK (5) later showed that these plants would reproduce under electric light, and investigated the growth in inorganic salt solutions under controlled conditions of light and temperature.

SAEGER (11) confirmed the results of CLARK and ROLLER by growing *Spirodela polyrhiza* in a diluted KNOP's solution without adding organic matter, but pointed out that auximones might have been added as an impurity in the salts or supplied by bacteria present in the culture solutions.

* Contribution from the Department of Chemistry, Iowa State College.

In an excellent summary of the auximone question WOLFE (12) reports work done in 1922 on *Lemna minor* with series I of the three salt solutions advocated by the National Research Council. With many of the solutions he obtained good growth without loss of health over a period of thirty-six days, but failed to get any increase by the addition of small amounts of various pure organic substances.

WOLFE considers that there is now sufficient proof to show that the failure of BOTTOMLEY'S plants to maintain normal growth in purely inorganic solutions was due to lack of physiological balance, and in consequence, auximones—meaning organic substances essential for the growth of the green plant—do not exist and the name should be dropped. He points out, however, that the question of stimulation by organic substances is still open, and further work is needed.

Methods

The experiments reported here were made in part during the winter months and the plants grown under electric light as described previously, CLARK (5). The *Lemna* cultures used have been transferred regularly in salt solutions for three years and have received no organic matter; the plants are healthy in appearance and color. The stock solution contains 0.4 millimoles of calcium per liter added as mono-calcium phosphate, 8 millimoles of potassium added as potassium nitrate, 1 of magnesium as magnesium sulphate, with 0.01 of iron as ferric chloride. The hydrogen ion concentration of the solution may vary slightly, depending upon the amount of phosphoric acid in the mono-calcium phosphate. Several different preparations of this salt were used: Baker's Analysed prepared for the National Research Council; the commercial Baker's Analysed; Kahlbaum's; and a sample specially purified by a method to be reported later. To prevent the addition of auximones, the salts were recrystallized three times, drying each time by centrifuge. In some cases Kahlbaum's Eisenchlorid—Zur Analyse mit Garantieschein—was used, instead of recrystallizing the C. P. ferric chloride. The water was three times distilled: the laboratory distilled water was treated with alkaline permanganate and redistilled; this was distilled a third time in a Pyrex glass apparatus, the steam passing through two traps before being condensed.

The *Lemna* were grown in 250 cc. Pyrex beakers which were covered with Petri dishes to prevent the entrance of dust. When the plants became crowded, about a dozen were left and the remainder discarded. The beakers were placed in a water bath at 25° C., and the water was kept low enough in the beakers to allow air to enter under the covers.

Results

The first experiment was run under electric light with an average intensity at the surface of the solution of 850 foot-candles, with the plants in the light 15 hours a day. The solutions tend to become more alkaline as the *Lemna* reproduce; they were therefore changed daily, and the plants were not allowed to fill the beakers. Under these conditions the change was not greater than 0.1 P_H in the 24 hours. The hydrogen ion concentration was varied in three series with $\text{Ca}(\text{OH})_2$, KOH , and NaOH . The amounts added were from very dilute solutions of the bases and the additions did not appreciably alter the concentration of the cation in the solution. The reproduction constant K was obtained as before, CLARK (5), from the equation

$$\log N - \log N_0 = K(t - t_0)$$

where N is the number of plants at any time t . Table I shows the P_H and the constant K ; fig. 1, A, the curve for K plotted with P_H as abscissa. A check solution, P_H 4.5 to 4.6 gave a value for K of 0.123.

TABLE I
GROWTH-CONSTANT K AND P_H OF SOLUTIONS. LIGHT 850 FOOT-CANDLES

P_H	K		
	KOH	NaOH	$\text{Ca}(\text{OH})_2$
4.8	0.148	0.151	0.151
4.9	0.133	0.134	0.142
5.0	0.122
5.1	0.114	0.111	0.106
5.3	0.100	0.104
5.4	0.083
5.6	0.079	0.078

Evidently an optimum P_H for this solution is between 4.5 and 4.8.

A second series was therefore run some time later under the same conditions except that the intensity of the light was smaller and the sodium hydroxide was not used. Growth started at P_H 4.2 and 4.3 but within two weeks the plants were small and many were brown tipped. The solution P_H 4.4 supported growth for some time longer, but the *Lemna* plants were poorly colored and reproducing irregularly at the end of three weeks. At P_H 4.5 the reproduction slowed down after the first ten days, but thereafter growth was regular at the reduced rate of speed. Table II gives the P_H and K .

TABLE II

P_H	K	
	KOH	$Ca(OH)_2$
4.5	. .	0.090
4.6-7	0.121	0.120
4.8	0.123	0.122
5.0	. . .	0.100
5.1	0.094	. .
5.3	0.085	. . .

Fig. 1, B, gives the curve for K and P_H . This curve, while lying below that of fig. 1, A, as expected from the previous work of CLARK (5), showed a distinctly optimum P_H around 4.7 to 4.8. Mr. ROLLER has indications that this optimum holds for the plants grown in daylight with the same solution, but that the greater acidity has less toxic effect.

Although the optimum P_H holds for this solution, a small change in the proportion of the salts will upset the medium. Three solutions were made up with varying quantities of the same salts, with iron as before, and the P_H brought to 4.7 with KOH:

- No. 1. Ca 0.8, K 8.0, Mg 0.8, millimoles per liter
- No. 2. Ca 0.8, K 4.0, Mg 0.8, millimoles per liter
- No. 3. Ca 0.4, K 8.0, Mg 0.4, millimoles per liter

In no case did the plants do well under either daylight or electric light, the fronds gradually becoming smaller and some of them taking on a brownish tinge. DEUBER (7), growing *Spirodela* in a diluted KNOP's solution and using iron as potassium ferrocyanide, found that the best growth was at an initial P_H of 6.2 and 6.8, but as the plants were changed only once in four days the solution increased as much as 0.9 P_H . The hydrogen ion concentration of a soil solution in which the plants make excellent growth was 6.3 P_H . The indications are therefore that the optimum P_H varies with the composition of the solution.

The *Lemna* grown in the more alkaline solutions lost color as if iron could not be assimilated. Ferric citrate added instead of ferric chloride did not improve the chlorosis nor the rate of growth.

The daily change of solution as used in these experiments, instead of three or four days as with BOTTOMLEY and DEUBER, or weekly as used by SAEGER, besides keeping the hydrogen ion concentration more uniform, prevents any large increase of bacteria or other life forms. Up to the present these plants, which reproduce by budding and not by flowers, have not been

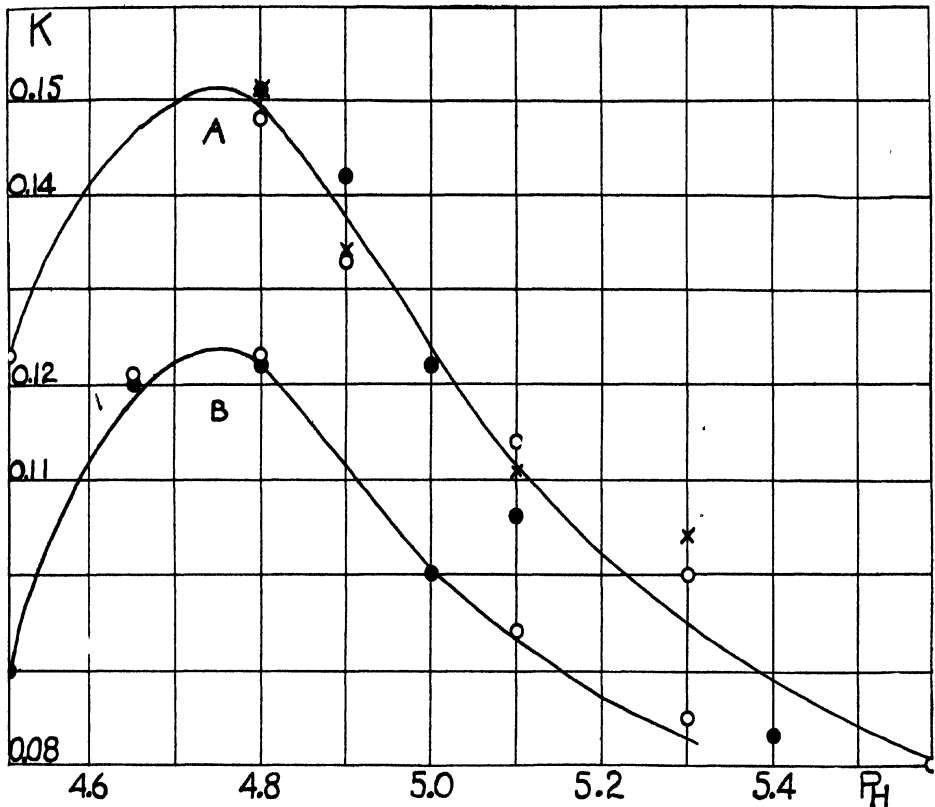


FIG. 1

A. Rate of reproduction K of *Lemna major*, with varying P_H . Intensity of light = 850 foot-candles.

B. K with varying P_H . Smaller intensity of light. P_H regulated with: \circ = KOH; \times = NaOH; \bullet = $\text{Ca}(\text{OH})_2$.

sterilized and cultivated completely free of bacteria. However, they grew well in a frequently changed solution which had been previously brought to boiling and made up to the original concentration with sterile water—a procedure which kept the bacterial content as low as possible.

In actual practice, the possible increase in the rate of growth of the plants due to increase in the numbers of bacteria or algae, when considerable time elapses between changes of the solution, is more than offset by other influences, for example, the alteration of the P_H of the solution due to the reproduction of the plants. A series of solutions was run with different time of changing, from 12 hours to six days, with the result shown in table III.

TABLE III
REPRODUCTION OF *Lemna* AND TIME OF CHANGE OF SOLUTIONS

TIME OF CHANGE IN HOURS	GROWTH CONSTANT K
12	0.098
24	0.093
48	0.088
72	0.084
96	0.081
120	0.074
144	0.069

At the end of three weeks the plants in the last three solutions were showing the effects of the long periods without change; the size was decreasing and the color was poor. These three were then discontinued, but the remainder were run for another two weeks. The experiment was repeated with a solution containing ferric nitrate and resulted in the same slight drop in the rate of growth as the time of change increased.

I wish to thank Mr. EMERSON R. COLLINS for checking the P_H values both by colorimetric and electrometric methods.

Summary

1. *Lemna major* grows and reproduces indefinitely in purified inorganic salt solutions without the addition of organic matter, in daylight and under electric light.

2. The optimum P_H for the solution used was from 4.7 to 4.8.

3. There are indications that this optimum varies with the composition of the solution, for a soil solution will grow good plants at a P_H which produces very poor and slow growing plants in the inorganic solution used.

4. Frequent changes of the solution, which lessen the amount of bacterial growth, increase the rate of reproduction of the plants. This does not show that bacterial products are unfavorable to growth, but that other influences are usually predominant.

5. Good growth was obtained by boiling the solution and changing the plants frequently in order to reduce the contamination by bacteria to a very small amount.

DEPARTMENT OF CHEMISTRY,
IOWA STATE COLLEGE,
AMES, IOWA

LITERATURE CITED

1. BOTTOMLEY, W. B. The significance of certain food substances for plant growth. *Ann. Bot.* **28**: 531-540. 1914.

2. ————. Some effects of organic growth-promoting substances (auximones) on the growth of *Lemna minor* in mineral culture solutions. Proc. Roy. Soc. London B **89**: 481–507. 1917.
3. ————. The growth of *Lemna* plants in mineral solutions and in their natural media. The effect of organic matter on the growth of various water plants in culture solutions. Ann. Bot. **34**: 345–365. 1920.
4. CLARK, NORMAN A. Soil organic matter and growth promoting accessory substances. Jour. Ind. Eng. Chem. **16**: 249–250. 1924.
5. ————. The rate of reproduction of *Lemna major* as a function of intensity and duration of light. Jour. Phys. Chem. **29**: 935–941. 1925.
6. CLARK, NORMAN A., and ROLLER, E. M. Auximones and the growth of the green plant. Soil Sci. **17**: 193–198. 1924.
7. DEUBER, C. G. Potassium ferrocyanide and ferric ferrocyanide as sources of iron for plants. Soil Sci. **21**: 23–26. 1926.
8. INDEX KEWENSIS—Oxford 1895. **2**: p. 50. *Lemna major* = *polyrhiza*. (GRIFFITH. Notulae ad plantas Asiaticas **3**: 216.)
9. MOCKERIDGE, F. A. The occurrence and nature of the plant growth-producing substances in various organic manurial composts. Biochem. Jour. **14**: 432–450. 1920.
10. ————. The formation of plant growth-promoting substances by micro-organisms. Ann. Bot. **38**: 723–734. 1924.
11. SAEGER, A. The growth of duckweeds in mineral nutrient solutions with and without organic extracts. Jour. Gen. Physiol. **7**: 517–526. 1925.
12. WOLFE, H. S. The auximone question. Bot. Gaz. **81**: 228–231. 1926.

DIFFERENCES IN RESISTANCE TO LOW TEMPERATURES SHOWN BY CLOVER VARIETIES

GEORGE STEINBAUER

(WITH TWO FIGURES)

Introduction

Differences shown by clover varieties in ability to withstand low temperatures are of great economic importance in the northern part of the United States. In this region the relative hardiness of a variety will often determine whether or not it is suitable for a given locality and whether or not it will endure average winter temperatures. The temperature limits for many crop plants can be determined best by field trials. Under field conditions, however, it is impossible to regulate or to control many conditions under which such tests are carried out and consequently the importance of a number of factors cannot be determined. Temperature is one of these factors. By mechanical control of temperatures it is possible to determine the response of varieties to different temperatures and the conditions under which the freezing of plant tissue occurs.

The aim of this work was to determine if relative hardiness of clover varieties could be determined under conditions where temperature could be controlled. Tests were carried out at different stages of growth with a number of varieties of clover and a few varieties of alfalfa. Differences in exposure of plants were eliminated as far as possible by air circulation. Care was taken to obtain uniform conditions during periods of hardening and exposure to low temperature. The following points were considered in this group of tests: (1) Effect of low temperature on germination of clover seeds before planting; (2) Effect of low temperatures on the germination of seeds which had imbibed various amounts of water; (3) Effects of low temperatures on plants of different ages; (4) Differences in resistance to low temperatures shown by a number of varieties in the seedling and mature stages of growth.

Results

EFFECT OF LOW TEMPERATURES ON THE VIABILITY OF CLOVER SEEDS

Clover seeds which had been exposed to laboratory conditions and had a water content of less than 15 per cent. by weight were subjected to temperatures ranging from 0 to -48° C. for 30 minutes. Germination tests before and after exposure to the lowest temperature were made, but showed no loss of viability from freezing. Dry seeds or those having low moisture

content are not injured by low temperatures ordinarily obtainable in the laboratory.

Low temperatures showed more harmful effects on the viability of clover seeds with higher moisture content. Seeds of Minnesota Medium Red clover with a germination of 90 per cent. were placed in distilled water for three to four hours at 37° C. At the end of that time they had imbibed approximately 90 per cent. of water, calculated on the dry weight basis. The results in table I show the percentage of germination after exposure to temperatures ranging from 0° C. to — 40° C.

TABLE I

INFLUENCE OF LOW TEMPERATURE ON GERMINATION OF MOIST CLOVER SEEDS

TEMPERATURE IN DEGREES C.	GERMINATION AFTER EXPOSURE IN PER CENT.
0	85.0
- 2	74.0
- 4	53.0
- 6	33.0
- 8	26.0
-10	9.0
-15	8.5
-20	5.5
-25	4.0
-30	0.0
-40	0.0

In another trial fully imbibed seeds were placed over a desiccating agent (H_2SO_4) of various known concentrations and the moisture partially removed. These were then exposed to various temperatures for 30 minutes and germination tests again determined. Germination fell off rapidly when the moisture content became greater than 15 per cent., as is shown by table II.

TABLE II

INFLUENCE OF MOISTURE CONTENT ON INJURY BY FREEZING

MOISTURE CONTENT IN PERCENTAGE	GERMINATION PERCENTAGE AFTER EXPOSURE FOR 30 MINUTES TO			
	- 10° C.	- 20° C.	- 30° C.	- 40° C.
89.75	9.0	5.5	0.0	0.0
25.	85.0	43.0	10.5	9.0
15.	85.0	55.0	50.0	43.0
13.	90.0	90.0	90.0	90.0
12.	90.0	90.0	90.0	90.0
10.	90.0	90.0	90.0	90.0

In fig. 1 are shown the effects of varying moisture contents on the killing points of clover seedlings. It is evident that as soon as the seed begins to imbibe moisture, it becomes much less resistant to low temperature.

EFFECT OF LOW TEMPERATURES ON PLANTS OF DIFFERENT AGES

The apparatus used for hardening and freezing seedling and mature plants consisted of a constant temperature chamber with electrical control. Low temperatures were secured by refrigeration and could be controlled within a range of .5 degrees C. Equal temperatures throughout the chamber are secured by means of an electric fan. Etiolation of the plants was prevented by the use of artificial light over the hardening chambers.

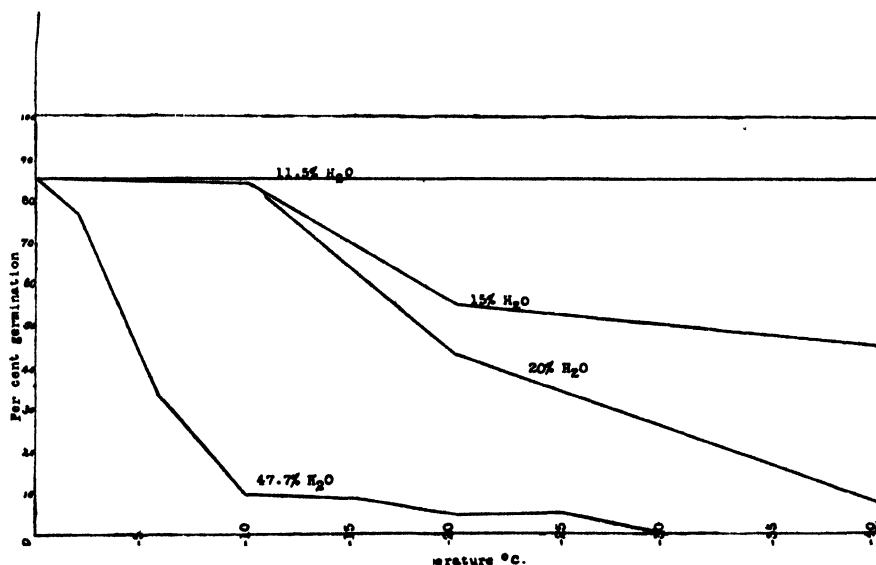


FIG. 1. Killing temperatures of clover seed (Minnesota Med. Red) with different moisture contents.

The necessity of having the plants for a given test at the same age is apparent, as there is considerable variation in the resistance to low temperatures of plants of the same variety at different ages. Difference in this respect is indicated by comparing plants of Minnesota Medium Red clover at various ages when exposed to low temperature, table III and fig. 2.

The plants seem quite resistant when emerging in the seedling stage and are most susceptible to frost injury when about three weeks old at the time of forming the first pair of permanent leaves.

Placing the plants at temperatures slightly above 0° C. for one or two days before freezing increased to a marked extent the ability of the plants

TABLE III
AGE AND RESISTANCE TO FREEZING

AGE	PERCENTAGE OF PLANTS KILLED AT -3°C .
7 days	0.0
10 "	17.0
14 "	46.6
21 "	100.0
90 "	0.0

to endure freezing temperatures, growth during this hardening period being materially checked. By so treating the plants it was possible to use lower temperatures and accordingly bring out more striking differences in cold resistance and in the rate of acquiring hardiness.

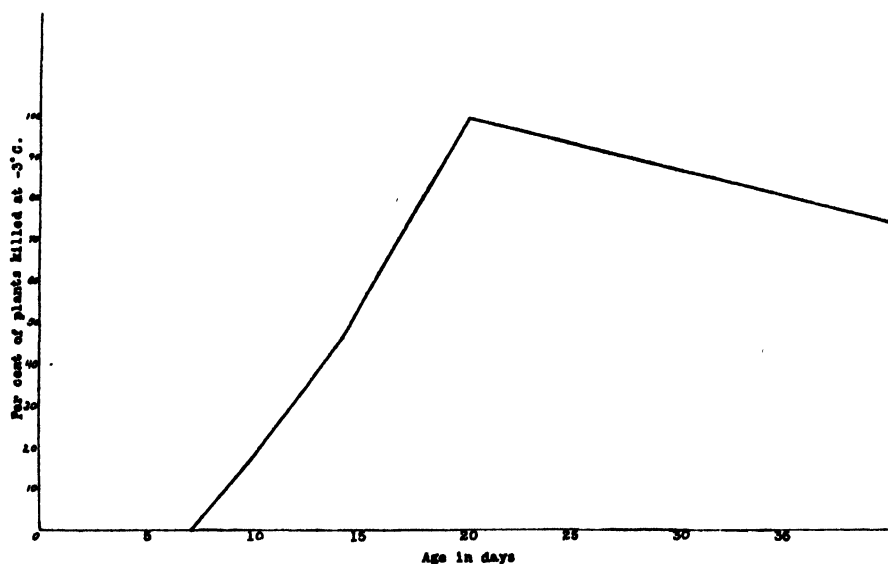


FIG. 2. Variations in low temperature resistance by clover plants of different ages (Minnesota Med. Red).

DIFFERENCE IN LOW TEMPERATURE RESISTANCE AMONG VARIETIES

Results obtained by hardening clover seedlings and then subjecting them to freezing temperatures showed considerable difference in cold resistance among the common varieties. Seeds were obtained through the courtesy of Dr. A. J. PIETERS, of the Office of Forage Crop Investigations, Bureau of Plant Industry. Only four varieties were used to simplify the trial. Duplicate tests gave results as shown in table IV.

TABLE IV
VARIETAL RESISTANCE OF CLOVERS TO FREEZING

VARIETY	PERCENTAGE ALIVE AFTER EXPOSURE TO			
	-4° C.	-5° C.	-6° C.	-7° C.
Altaswede	100	100	85	30
Michigan	97	94	83	30
French 2213	86	80	52	19
Italian 54779	92	64	70	15

The seedlings were seven days old when hardened. Freezing was observed at higher temperatures when the plants were in contact with water. Freezing of seedlings in vials showed injury to occur first on the root end of the seedling.

With more mature plants the results were similar to those obtained in the seedlings. In general the northern varieties were more resistant and responded more rapidly to hardening. In tables V and VI are given data from two of many sets of clovers showing the approximate percentage of killing. The plants used were three weeks old and hardened for about 48 hours at 2° C. before trial.

TABLE V
VARIETAL RESISTANCE OF CLOVERS TO FREEZING

VARIETY	PERCENTAGE KILLED AT -4° C.
Minnesota Med. Red Clover	50.
Ohio Red 1687	60.
French Red 1233	60.
French Red 1247	65.
Bohemian 1250	80.
Idaho Red 1287	92.
Hungarian Red 1237	95.
Chilean Red 1285 (one plant in 25 unin- jured)	96.
French Red 1291	100.
Australian 1655	100.

In all cases where plants were not completely killed the injury was evident first on the outer leaves, whereas crowns were less easily injured.

TABLE VI
VARIETAL RESISTANCE OF CLOVERS TO FREEZING

VARIETY	PERCENTAGE KILLED AT -4° C.
Minnesota Med. Red Clover	40.
Oregon Red 2148	50.
French Red 1294	55.
Ohio Red 1687	60.
Idaho Red 1287	65.
Chilean Red 1239	80.
Chilean Red 1605	93.
Tennessee	95.
Australian Red 1655	100.
French Red 1291	100.
Bohemian Red 1250	100.
Chilean Red 1285	100.
Hungarian 1237	100.

Summary

1. The viability of clover seeds containing a moisture content of less than 15 per cent. of the dry weight is unaffected by temperatures as low as -48° C. for short periods.

2. The germination of clover seeds with high moisture content is greatly impaired by low temperatures, falling off very rapidly in seeds with a moisture content above 25-30 per cent.

3. Clover plants of the same variety vary with age in respect to killing temperatures. Susceptibility to injury is most pronounced at about three weeks of age, or at the time of formation of the first pair of permanent leaves.

4. There is considerable difference among varieties in ability to withstand low temperature. In general European or southern varieties proved less resistant than those grown in northern areas of the United States. This variation was present both in the seedling and mature plants.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
 UNIVERSITY OF MINNESOTA,
 ST. PAUL, MINNESOTA

BRIEF PAPERS

FOOD STORAGE

The amount and compactness with which storage substances are deposited in certain cells varies to a considerable extent. In some instances it appears as if the cells concerned are so densely filled that no available space is left, but in others this is less pronounced. The amount of unused space, however, is generally greater than direct observation would indicate. The writer has determined this for individual cells and in different plants by means of centrifugal force of varying intensity and lengths of application. In a previous study of this subject a few seeds of *Vicia sativa* were found whose cells were so compactly filled that displacement did not occur when acted on by 4400 gravities. The soaking of most seeds and other storage structures, which have become dry, is necessary to allow displacement by this means. An exception to this is shown by fresh seeds, for example, in whose cells oil occurs. Even here, as would be expected, desiccation must not progress too far. Soaking from 12 to 24 hours or more is generally necessary and due regard to temperature is advisable. Since a rise of temperature often occurred due to air friction in the drum of the apparatus used, provision for this condition entered into the experiment. In many of the experiments 5000 gravities or more were used for two hours or more and in all cases the storage structures were enclosed in strong glass cylinders. In some instances the force applied varied from 2000 to 3000 gravities and in such cases was frequently applied continuously for 24 hours or more. A force of this intensity and duration was frequently effective in displacing the dense contents of certain storage cells, whereas its action was not noticed when continued for only a short time. In certain storage structures only a few gravities produces displacement, while in some cells gravity alone will cause starch grains to fall to the lower side of the cells as shown by DEHNECKE and others. The difference in compactness of the solid storage substances, as well as the size of the cell, influences the speed and ease of the displacement of the cell contents. This is shown in the tubers of *Solanum* in which the starch grains may fall to the lower side of certain cells in a few minutes, while in very small and more densely filled cells the transit of the starch grains may require 45 minutes or more. The cell contents of freshly gathered acorns of *Quercus* showed a slight displacement with 3000 gravities acting for five hours. For example, in the following three species, *Quercus alba*, *Q. macrocarpa* and *Q. rubra*, the displacement amounted to about 1/10, 1/15, and 1/12 respectively of the total cell volume. When, however, roots three cm. long were produced, displacement occurred

in certain cells varying from $1/5$ to $1/4$ of their volume. Even before growth, when placed at an angle of 90 degrees to the line of centrifugal force, the contents were about re-distributed in from 15 to 30 days. Slight displacement only was observed when 3000 gravities acted on the seeds of *Aesculus Hippocastanum*, but a longer time was required for re-distribution of the cell contents during germination than in *Quercus*. Seeds of *Pisum sativum* subjected to 5800 to 6000 gravities and acting for three hours showed that many of the cells were about $1/3$ filled with starch grains. The heavier contents in the seeds of *Ricinus*, *Helianthus* and *Cucurbita* when 5800 to 6000 gravities were used were driven to the centrifugal end of the cells and occupied about $1/3$, $1/4$ and $1/5$ of the volume of the cells respectively. The compacting of the storage materials in these experiments was somewhat more pronounced than was the case when 4400 gravities were used, as in my previous experiments. The lighter substances passed to the centripetal end of the cells and filled the remaining space. With these, as in my former experiments, no separation of the protoplasmic membrane from the cell walls occurred, nor were the cells killed by the long continued action of this strong force. A further study of this subject is in progress.—
F. M. ANDREWS, *Indiana University, Bloomington, Indiana.*

NOTES

Officers for 1926-1927.—The results of the annual election of the American Society of Plant Physiologists have been announced, as follows: President, Professor FRANCIS E. LLOYD, McGill University, Montreal, Canada; Vice-President, WRIGHT A. GARDNER, Alabama Polytechnic Institute, Auburn, Alabama; Secretary-Treasurer, SCOTT V. EATON, University of Chicago, Chicago, Illinois.

Professor LLOYD is of Welsh ancestry, and was born at Manchester, England, in 1868. He was graduated from Princeton University in 1891, and received his A.M. degree from the same institution in 1895. Afterwards he was a student at Munich and Bonn. He has had a wide experience as an investigator, and has been for a number of years MacDonald Professor of Botany at McGill University. His recent studies of chlorophyll fluorescence, the conjugation behavior of *Spirogyra*, and the habits of *Vampyrella* have attracted much attention.

International Congress of Plant Sciences.—The editor regrets that this issue of PLANT PHYSIOLOGY will be delayed beyond the meeting of the Congress at Cornell University, Aug. 16-23, 1926. The preliminary announcements indicate that the meetings will be of great value. It is a great privilege to American botanists to be hosts to the visitors from other lands, and success of the great undertaking has been possible only by the splendid cooperation of the various committees and secretaries of the various sections.

Arrangements have been made to give a detailed account of the Congress to our readers in the October number of PLANT PHYSIOLOGY, particularly the physiological proceedings.

Regional Meeting at St. Paul.—About thirty-five members of the Society met at St. Paul in conjunction with the Corn Belt Section of the American Society of Agronomists, July 15-17, 1926. Some of the meetings, especially the joint meetings, were held in the field, while the special physiological meetings were held in the physiological laboratories at the University of Minnesota Farm. The program consisted mainly of demonstrations of apparatus and methods of research with light of various kinds, monochromatic and polarized; electrical conductivity; thermoelectric cryoscopy; ethylene treatment of fruits and vegetables; preservation of all kinds of fruits and vegetables in their natural colors; apparatus for continuous respiration, etc. Everyone in attendance found the meetings full of interest and inspiration.

The Minnesota Section.—The American Society of Plant Physiologists has approved the establishment of a Section at the University of Minnesota. Permanent officers have not yet been chosen, but permanent organization is to take place in the near future. Minnesota has a large group of men interested in physiological research, and has its laboratories well equipped for such investigations. The organization of a Section there should stimulate a strong spirit of cooperation, and add life and inspiration to the work. We wish the Section success in all of its undertakings, and hope that it will be a strong link in the chain of sections to be established as the Society progresses.

An Endowment Fund.—Should the American Society of Plant Physiologists establish a small endowment fund to which members could contribute whenever they felt able to do so? The time may not be ripe for such a movement now, but it is worth while giving it some thought. Every organization finds itself better able to perform its legitimate tasks if it has sound financial resources represented by reserve funds accruing from life memberships and gifts for endowment. It is possible that arrangements could be made for the duplication of small gifts up to a total of several thousand dollars, if the Society authorized the establishment of such a fund. Would it not be worth while to consider the advisability of such a move at some general meeting of the Society?

The Program Committee.—One of the standing committees of the Society is the program committee. The meeting at Philadelphia next December will be a notable one, and it is not too early to begin laying our plans, and arranging the program for the third annual meeting. The members of the Society can facilitate the work of the committee by early preparation of titles and abstracts, so that the committee and the secretary will have a choice of material for well arranged, systematized meetings. The enthusiastic support of the programs at the Kansas City meeting was an inspiration to all who attended, and augurs well for all future meetings of the Society. The program committee has been named by the President, Prof. FRANCIS E. LLOYD, of McGill University, and consists of the following members: Dr. A. E. Murneek, Univ. of Missouri, chairman; Dr. Lee M. Hutchins, Bureau of Plant Industry, U. S. Dept. of Agr., and Dr. W. F. Gericke, Univ. of California, Berkeley. Hearty and loyal cooperation with the general officers of the Society and the program committee will insure a noteworthy gathering of Plant Physiologists, and a valuable program for the convocation week meetings.

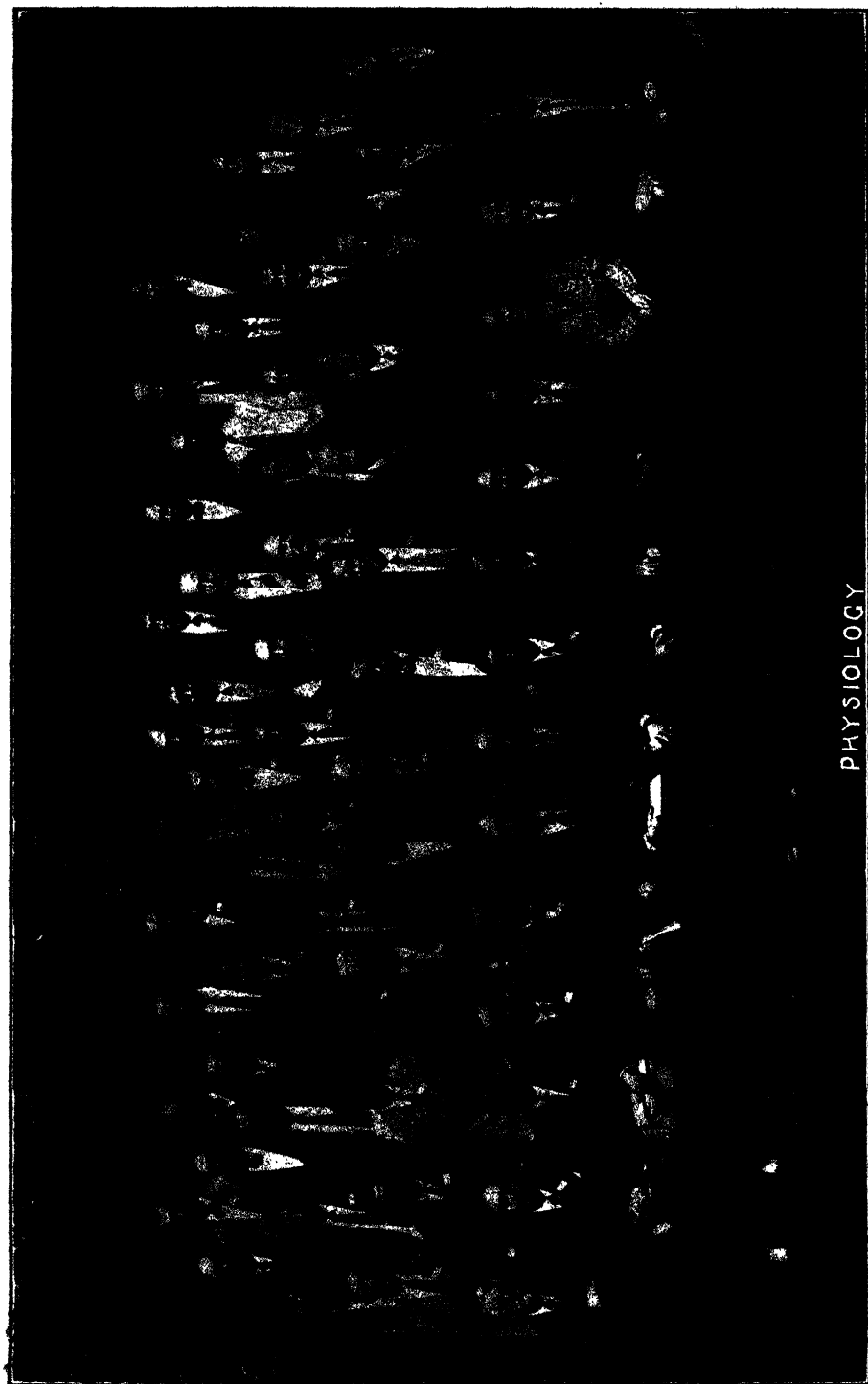
Committee on Analytical Methods.—The committee on analytical methods of the American Society of Plant Physiologists is engaged in a study of the methods of analysis suitable to plant materials, with special reference to the simple carbohydrates, the simple polysaccharides, simpler nitrogenous constituents, and the proteins and basic forms of nitrogen. The committee consists of Dr. W. E. Tottingham, Univ. of Wisconsin, chairman; Dr. W. E. Loomis, Univ. of Arkansas; Dr. J. J. Willaman, Univ. of Minnesota; and in advisory capacity, Dr. T. G. Phillips, New Hampshire Agr. Exp. Sta., and Dr. C. O. Appleman, Univ. of Maryland. Plant Physiologists have long needed a critical study and review of the methods of analysis such as is being made by this committee. The members will look forward with much interest to the published reports of the committee, which should begin to appear within the next year.

International Critical Tables.—The first volume of this important work has been received from the press of the McGraw-Hill Book Co. It is published for the National Research Council, Washington, D. C., and will be complete in five volumes. This volume contains a number of sections, the first of which deals with systems of weights and measures, symbols, constants, conversion factors, atomic numbers, and atomic weights, periodic system, isotopes, and atomic structure. The second section deals with laboratory technique, thermometry, maintenance of constant temperatures, humidity, psychrometry, weighing, calibration, buffers and indicators, high vacuum technique, and errors of observation. The main body of this volume is made up of ready reference tables of physical properties of chemical substances. There are also sections on radioactivity, astronomical and geodetic data, and aerodynamics. The tables will be immensely valuable to investigators in the physical and biological sciences.

Nervous Mechanism of Plants.—A new book by BOSE, published by Longmans Green and Co., bears this title. It contains seventeen chapters, and attempts to support the thesis that plant response is governed by a system of "nerves" similar physiologically to animal nerves. The author identifies the "nerves" of *Mimosa* with the phloem strands, which occur as inner and outer phloem in a bicollateral bundle, and he believes that phloem generally acts in plants as the transmission medium of excitation. The cross walls are believed to act as synaptic membranes, and a close comparison is made between animal and plant behavior under electrical and other stimuli. If we were simply to refer all of the phenomena observed to general protoplasmic conduction of stimulus, there would be little to criticize in the presentation. But there is nothing in the structure of the plant as we now

know it to indicate anything beyond general sensitivity and conductivity of protoplasm. It would be unfortunate to add a nervous mechanism to the popular misconceptions of plant life.

Physiology of Plants.—A textbook of rather elementary grade has been written by PEIRCE, who treats the subject in ten chapters, four of which are on nutrition, and two on irritability. Other chapters deal with movement of water, metabolism, and reproduction. There seems to be too much round about discussion, and not enough data for a most useful textbook. It does not materially relieve the situation with respect to textbooks in English for students of plant physiology. It is published by Henry Holt and Co., New York.



PLANT PHYSIOLOGY

OCTOBER, 1926

PLANT PHYSIOLOGY AT THE ITHACA CONGRESS

FRANK THONE

The International Congress of Plant Sciences held at Ithaca, N. Y., August 16 to 23, 1926, was the botanical event of a lifetime to those who were fortunate enough to be present; and it is safe to assert that no group came away with more stimulating and provocative recollections of the week than the plant physiologists. Several of the papers there presented were wholesomely upsetting to accepted notions, others were helpful in the clearing up of concepts hitherto somewhat foggy, or summed up knowledge hitherto incomplete or scattered. The program committee is to be congratulated for its complete avoidance of those ten-minute soporifics that sometimes creep in despite the best-laid plans.

It was regretted by everyone that the chairman of the physiological section, Prof. LEPESCHKIN, of Charles University, Prague, could not be present to preside at the meetings. In his absence Dr. C. O. APPLEMAN, University of Maryland, presided over the discussions.

The color of the entire meeting was indicated by the first paper of the opening session, on the osmotic properties of the plant cell, by Prof. A. URSPRUNG, of the University of Fribourg, Switzerland. Prof. URSPRUNG was unable to attend in person, and arrangements had fortunately been made to have this communication read by an American associate, Prof. WILLIAM A. BECK, of the University of Dayton. Prof. URSPRUNG's paper belonged to the "upsetting" category, and was received alertly, but in an atmosphere of general caution. It was recognized that if his results are fully borne out by further work, large sections of current standard physiological interpretation in the field of water absorption and translocation will have to be radically revised.

Prof. URSPRUNG has greatly refined the technique for studying osmotic phenomena, both in improvements in manipulation, and in the substitution

of sugar solutions for the potassium nitrate or similar salt solutions now in vogue for plasmolytic studies. He further insists that the critical point in plasmolysis is not the moment of incipient withdrawal of protoplasm from the wall of the cell, but at the beginning of diminution of distention, the first signs of shrinkage, of the fully turgid cell. Determination of plasmolytic concentrations by this method leads to radically higher values for the suction tensions in plant cells than have so far been commonly reported.

He introduces several new terms into the discussion, differentiating between the suction force of the contents of the cell, wall pressure, turgor pressure, and the suction force of the cell. The relations of three of these terms are indicated by the equation:

$$(\text{Suction force of the cell}) = (\text{Suction force of the contents}) - (\text{Wall pressure}).$$

The quantities are notionally and numerically different, and must not be designated by a common term, e.g., "osmotic pressure"; furthermore, each quantity varies with the degree of saturation of the cell.

In studies on the translocation of water, a very steep gradient from root hairs to uppermost transpiring organs (leaf palisade, petals) was discovered. This gradient held not only for entire plants but also for their parts. Thus, in a row of palisade cells in an ivy leaf the minimum suction force (12.1 atmospheres) lay near the vein, and an increase was noted with the distance from the vein, up to 32.6 atmospheres.

Perhaps the most outstanding of Prof. URSPRUNG's discoveries relates to the existence of a polarity of suction forces exerted by opposite sides of the same cell, and the effect of this polarity in certain cells of the root in carrying forward the unilateral movement of water in the plant as a whole. It was discovered that in a cross section through the zone of absorption of the root tip of *Vicia Faba*, the suction force rose from 1.1 atmospheres in the epidermis to 4.1 atmospheres in the innermost cells of the cortex, but then suddenly dropped in the endodermis to 1.9 atmospheres. In spite of its low mean suction force, the endodermis can still absorb water by virtue of the polar differentiation of the suction force. On its outer side 4.7 atmospheres were found, and on its inner side 0.5 atmosphere. Thus the endodermis acts simultaneously as a suction pump and a reduction valve. Polar differentiation is even more marked in the parenchyma cells that border on the root vessels; like a combined suction pump and force pump they can take up water on one side and press it into the vessels on the other.

The influence of climatic and edaphic factors on the suction force was also studied. It was found that the moisture of the soil plays the chief role; a heavy rain caused the suction force of the leaf of *Satureia alpina* to fall from 34.5 to 13.5 atmospheres. The relative humidity of the air

was found to be next in importance; its fluctuations on a summer day caused the corolla of *Bellis* to vary by about 10 atmospheres.

If the proper precautions be taken, the influence of habitat on the suction force can also be determined. In the Alps, for instance, the vexillum of *Lotus corniculatus* showed 9.5 atmospheres in a moist locality, 14.5 atmospheres on the alpine meadow, 29.5 atmospheres on a belt of humus, and 34.5 atmospheres on dry rubble, simultaneously. The suction force increased in this case with the reduction of the water supply. A comparison of the suction force in the plain with that in the mountains did not manifest a clear difference, and SCHIMPER's idea of the physiological dryness of a peaty substratum could not be verified.

Dr. H. S. REED, of the Citrus Experiment Station at Riverside, California, attacked the vexing problem of the significance of quantitative expressions of plant growth and differentiation. Growth and differentiation, he said, may be considered as irreversible changes in the mass of an organism through which there is an increase in size and complexity. The transformation of extraneous materials into the mass of the organism appears to proceed at a rate proportional to time.

Our knowledge of the growth of organisms has been immensely advanced in recent years by the application of quantitative methods to its study. Having found that the increase in size of an organism is an orderly change, it is possible to give mathematical expression to it, analogous to those used to express chemical processes. Biologists have not yet settled on any one equation to express the process, perhaps none yet advanced is adequate for the purpose. Their use has, however, focussed attention upon the orderly nature of the growth process and makes it more apparent that this process is a manifestation of the energy relationships prevailing in other forms of matter. The size of a plant at a given time appears to depend on the nutrient level and on the specific constant of growth. The latter is independent of environmental conditions and expresses the velocity of transformation of unit mass of nutritive material into tissue substance. The growth curves of the same race growing at different rates easily allow one to determine the validity of these constants.

A brief experience with growth curves of organisms shows the prevalence of growth cycles, which are quite independent of definite fluctuations in environmental conditions. These cycles overlap to a certain extent and it is frequently impossible to discern their exact limits. The mathematical expression of growth cycles presents no especial difficulties whether one regards them as successive or as a single process, in which the main reaction is periodically accelerated or retarded by one of the products of the reaction.

A more precise study of the processes of differentiation and development has shown the existence of causal factors which produce a relative constancy of form of individuals of a given species or variety. The size of the organs bears evidence that the process of differentiation results in a stoichiometric distribution of mass with respect to space. The precise form is dependent upon the distribution of plastic materials, the dominance of the apical meristem, the release of certain buds from dormancy, all of which appear to be correlated. The biomathematical aspects of the physiological problems have become increasingly important.

Dr. V. LUBIMENKO, of the Leningrad Botanic Garden, offered a masterly summing-up of our present knowledge of the composition and genesis of chlorophyll, to which his own researches have been a major contribution. He holds that the food-building operations of plants began their evolution as chemosyntheses, similar to but simpler than those now familiar in certain bacteria. Photosynthesis replaced chemosynthesis after the formation of pigments in the protoplasm of saprophytic plants, the process becoming more complex as one ascends the evolutionary scale.

The formation and accumulation of chlorophyll in the algae, mosses, ferns, and gymnosperms is accomplished in the absence of light, which for these plants serves only as a stimulator. Among the angiosperms light enters directly into the reactions of the formation of chlorophyll; however, enzymatic reactions also play a part in the accumulation of chlorophyll in connection with the photochemical reactions. Leucophyll is probably a single definite albuminoid substance, and the formation of chlorophyll is intimately associated with the transformations of the albuminoid substances in the cell. The spectrocoulometric method is most rapid and most exact for the estimation of chlorophyll in living leaves. By means of this method it has been established that the maximum amount of chlorophyll elaborated by various species is a constant and represents an hereditary character. The application of illumination of feeble intensity is manifested by an increase of the pigments in the plastids; this phenomenon has been observed not only with terrestrial plants but also with marine algae. Among the red algae the quantity of phycoerythrin increases in greater proportion than the quantity of chlorophyll with the depth of habitation. The number of species rich in chlorophyll among land plants increases in the north and attains a maximum at the equator.

The hypotheses advanced by various writers on the chemistry of photosynthesis are inadequate, for they do not take into account completely the condition of the chlorophyll in the plastids. In every case it is certain that the synthesis of the organic materials in the plastid includes not only photochemical reactions, but also enzymatic reactions. This is the reason there is no simple proportionality between the energy of photosynthesis and the

quantity of chlorophyll in the leaves. A maximum energy of photosynthesis corresponds to an optimum quantity of chlorophyll. Under natural conditions it is low light intensity or temperature which is able to limit the energy of photosynthesis. In general plants do not suffer from insufficient CO_2 in the atmosphere, for the energy of the photochlorophyllous work of the leaves is conditioned and limited by internal factors, and notably by the speed of transport of the substances assimilated by the leaves during the day. Upon these internal factors depends the adaptation of the plants to intensity and periodicity of illumination.

It is very probable that it is the relative energy of oxidations and reductions which determines in the end the complete course of the organic syntheses in the plant cells. The energy of these enzymatic processes determines the physiological condition of the plastids, as well as the qualitative and quantitative evolution of the pigments. Therefore a searching study of the enzymic activity of the plastids promises to clarify the fundamental problem of plant physiology—the problem of photosynthesis.

Dr. LUBIMENKO showed specimens of aqueous chlorophyll extracts, which had proved to be stable through long periods of time. He also demonstrated his spectrocoulometric apparatus for the quantitative study of chlorophyll in the living plant.

Prof. B. E. LIVINGSTON, of Johns Hopkins University, addressed the section on the importance of viewing the environment, no less than the plant, as a dynamic complex. Physiologists have long been accustomed to look upon the plant as a mechanism receiving material and energy from its environment and delivering the material and dynamic products of its activities back to the environment. This view of the organism as solely active and the environment as solely passive is a one-sided picture. Matter and energy pass into the receptive plant by virtue of a potential with which they are already endowed, and the removal of by-products from the plant is also a function that is at least partly active. These positive physiological functions of the environment are susceptible of quantitative study, and beginnings in this direction have been made, especially in the examination of the evaporating power of the air, and of oxygen, CO_2 , and water supplying power of the soil.

On the afternoon of August 17, the physiologists, horticulturists, and geneticists met with the agronomists for a round table discussion of the place of statistics in the interpretation of experimental data. The leader of the discussion was Dr. H. H. LOVE, of Cornell University. Space will not permit presentation of the various points of view expressed on this important subject. Application of statistical methods to physiological research would often prevent the drawing of erroneous conclusions from inadequate data.

On Wednesday morning, in the absence of Prof. LEPESCHKIN, the discussion was opened by Dr. W. J. ROBBINS, of the University of Missouri, who discussed the isoelectric points for plant protoplasm and their significance. The paper was an excellent summary of the recent work done on isoelectric points in living tissues, much of which has been done in the Missouri laboratory. Following this paper, Dr. L. MICHAELIS, of the University of Berlin, presented a brilliant paper on permeability of vegetable membranes and an artificial model for these membranes before an audience that taxed the meeting place to the limit. The summary of this paper was prepared for PLANT PHYSIOLOGY by Dr. MICHAELIS, and is presented in his own words.

“It has been shown by JACQUES LOEB and R. BEUTNER that the integuments of many plants in contact with electrolyte solutions establish an electric potential difference, the amount of which depends on both the nature and the concentration of the dissolved electrolytes. This effect can be most easily studied on the intact peel of an apple, but the leaf of *Plantago* also may be recommended. The effect is due to the intact wax cuticle of the surface. The P.D. depends on the concentration of a given electrolyte, in that, by a change by one power of 10 of the concentration of any electrolyte the cation of which is univalent, it will produce a change of the potential by 57 millivolts as a maximum. Such a skin therefore behaves like a metallic electrode reversible for the cation of the electrolyte dissolved in the aqueous solution.

“BEUTNER tried to explain this effect according to HABER’s theory of potential differences at interfaces of different phases. However, the explanation is easier on the basis of a certain artificial model of membrane which gives the same effect in any regard, and can only be explained by the theory of diffusion-potentials. This model is a membrane of completely dried collodion. It can be shown that this is approximately impermeable for any anion. The cation can penetrate the membrane, but the specific velocities of the different sorts of cation are much more different from each other than in an ordinary aqueous solution. The greatest difference of velocities for aqueous solutions is the difference in mobility of H^+ and Li^+ (about 10 to 1). Within the pores of the collodion membrane this ratio of velocities sometimes amounts to 1000 to 1. There is even a considerable difference in the mobilities of K^+ and Na^+ .

“Diffusion of electrolytes across the apple skin can practically take place only if cations can be exchanged across the membrane, but not when the apple is dipped into pure water. All of these properties depend on the negative electric charge of collodion, and the exceedingly small pore size. There is no other effect than one of a sieve the holes of which exceed the

molecular size by only a little. The permeability depends on the molecular size, the sign of charge, and the amount of water carried by the ion.

“The plasma membrane differs from this cuticle by the fact that the structure and pore size of collodion (apple skin, etc.) are practically independent of the surrounding solution, while the real plasma membrane can be easily changed (coagulated or peptized) by electrolytes, especially by calcium. The problem of the permeability of the plasma membrane consists therefore of two different problems:

“(1) How is the colloidal structure influenced by the surrounding solution?

“(2) What is the permeability of a membrane of a given structure for the different ions of the surrounding solution?

“The apple skin and the collodion model give us a nearly complete understanding of the second question. The problem presented by the first question, however, is not touched by these investigations. The combination of these two problems may give complete understanding of the permeability of the plasma membrane of living cells.”

The meeting of Wednesday morning closed with a discussion of the mechanism of stomatal movements. This has been one of the most fascinating subjects to plant physiologists. A great deal of work has been done on it, nevertheless it has remained somewhat of a mystery. Prof. G. W. SCARTH, of McGill University, has found a correlation between the movements of stomata as well as other plant cells and organs, and the hydrogen ion concentration. Leaf sections in solutions whose P_H was subject to control showed minimal stomatal opening at P_H 5.5, and changed little until the neutral point was reached, but then opened rapidly. There is also a correlation between amylolysis and degree of closure. Starch is abundant at the P_H of closure (maximum starch at about P_H 5.0) and it dwindles in amount if the guard cells become more acid, but especially on the alkaline side of neutrality. In spite of these correlations, however, synthesis and hydrolysis of starch do not wholly account for the stomatal movements; for movements occur with changes of P_H much more quickly than any change in starch can occur. Also, even when the starch is all hydrolyzed, still changes in P_H cause changes in turgor, and movements. Beautiful photomicrographs were used to show that the effective mechanism of movement is concerned with hydrophilic colloids of amphoteric nature in the guard cells, supplemented possibly by the hydrolysis and synthesis of the carbohydrates. Open stomata are alkaline in *Pelargonium*, and closed stomata acid. The alkalinity of guard cells during photosynthesis is believed to be due to the use of the CO_2 by the chloroplasts of the guard cells, and the acidity which causes closure is the consequence of higher CO_2 pressure in

the guard cell when photosynthesis ceases and respiration continues. Openings at night may be due to development of hyperacidity, which also causes hydration of the colloidal material involved in the production of turgor. The speed with which opening and closing takes place on access or exclusion of light indicates that under normal as well as under experimental conditions, the primary mechanism through which the H-ion increase or decrease acts, is the hydration of the vacuolar colloid rather than hydrolysis of starch. Thus this problem nears a complete physico-chemical solution.

On Thursday morning the physiologists met jointly with the horticulturists, and in the afternoon with the horticulturists and ecologists for a round table discussion of the factors influencing flowering and fruiting *versus* vegetation. Naturally much attention was given to the well known work of GARNER and ALLARD on photoperiodism, and the discoveries which have grown up out of their recognition of the powerful influence of the illumination period on flowering and other events in the life cycle of plants.

Professor AUCHTER, of the University of Maryland, discussed the influence of shading on the behavior of apple trees, and Prof. H. C. THOMPSON, of Cornell, presented a most interesting study of temperature effects on flowering of celery. Early chilling induced premature flowering of this crop. The physiological significance of carbohydrate accumulation as a main cause of dormancy was presented by Dr. H. D. HOOKER, of the University of Missouri. One of the finest papers presented was not listed on the program, a paper by Professor WALLACE, of the Long Ashton Station, Bristol, England, on the effects of mineral deficiency on the growth and leaf behavior, flowering and fruiting of fruit trees and woody shrubs. The Ca/K ratio was found to be important in controlling physiological breakdown of leaf tissues.

The round table discussion in the afternoon took up photoperiodism, the carbohydrate-nitrogen ratio, phosphorus, carbon dioxide enrichment, water supply, etc., as related to vegetative and fruiting activities of plants. It was featured by the presentation of data by Prof. R. H. ROBERTS, of Wisconsin, who showed that the behavior of the plant is closely correlated with the internal nutritive conditions, especially the carbohydrate-nitrogen situation.

Just as the first day's session opened with a paper of unusual interest, so also did the program of Friday, the last day of the Congress, begin with the presentation of data very disturbing to many of our cherished physiological and ecological ideas. Prof. N. A. MAXIMOW's paper left little place for the orthodox idea that xerophytes are plants of low transpiration rates and low water requirement. Many xerophytes, for instance, *Peganum*, *Zygophyllum*, and *Artemisia*, transpire intensely and possess a high water requirement. On the contrary, shade mesophytes lose water very slowly.

Observations on plants in the open have shown that on bright days in early summer xerophytes of the type mentioned above reduce the water content of their leaves in the afternoon. The decrease in the water content attains, according to the determinations of Mrs. T. KRASNOSSELSKY-MAXIMOW, 30 per cent. of the water reserve of the leaves. In the hottest and driest period the xerophytes have their leaves in a state of permanent wilting.

These observations induced Prof. MAXIMOW to study the phenomenon of wilting, and the relation to it of plants of different ecological types. It developed that xerophytes were much more enduring to wilting than mesophytes; and just in this capacity of enduring permanent wilting without injury lies the chief peculiarity of drought resisting plants.

The capacity of enduring wilting is not quite constant for each plant. It varies with the conditions of growth. Plants cultivated in moist air and in a moist soil become very sensitive to wilting. On the contrary, plants that have undergone severe wilting become considerably more tolerant of drought. This increase of drought resistance is combined with definite anatomical changes. The cells of the newly formed leaves become smaller, the number of stomata per unit area increases, and the venation becomes denser. A physiological consequence of these changes in structure is an increase of transpiration and assimilation.

Xeromorphic structure can be the consequence of dryness of the atmosphere, as well as that of the soil. Prof. ZALENSKY long ago showed that the upper leaves of a plant are always of a more xerophytic nature than the lower ones, and in accordance with this ALEXANDROV has shown that the upper leaves transpire and assimilate more intensely. Sun leaves are also more xeromorphic than shade leaves.

The xeromorphic structure reflects the changes in the physiological properties of the plant; it accompanies the increase in the osmotic pressure of the cell sap as well as the increase of the water-retaining capacity of the colloids of the plasma. This is just what makes the plants more tolerant of wilting. Besides, these anatomical peculiarities favor a better water supply when the amount of water in the soil is low, and therefore the xeromorphic plants can develop better in dry habitats.

Not all the xerophytes possess xeromorphic structure. Succulents, which are provided with a big water reserve, represent a special physiological type. Ephemeris, too, represent a peculiar type. Their anatomical peculiarities must not be considered in fixing the fundamental characteristics of a xeromorphic structure.

The widespread opinion that the bacteria and *Cyanophyceae* of hot springs may be relicts of the original plant population of the earth was combated by Dr. T. V. VOUK, of the Botanicki Zaved, Zagreb, Jugoslavia.

He bases his objections to this theory on the facts that thermophiles are phylogenetically heterogeneous and that practically all of them have close relatives widely distributed in cold fresh waters. His own experiments convince him that even the organisms inhabiting the hottest waters are still in a more or less plastic condition as regards adaptation to their peculiar environment. He believes that the present inhabitants of thermal springs migrated into the hot waters at different periods of the earth's history, though at this time it is impossible to say when, because as yet we have no facts whatever on which to base conclusions.

The difficulties of propagating plants from seeds and cuttings were discussed in a very illuminating paper by Dr. WILLIAM CROCKER, who gave an account of the work being done along this line at the Boyce Thompson Institute. Methods of stratification of seeds at 5° C., and of producing rooted cuttings of plants that seldom root, were described. Important progress has been made toward inducing rooting of cuttings of many kinds of woody plants.

The problem of the germination of orchid seeds and its relation to the supposed symbiotic fungi of orchids was discussed at length by Dr. LEWIS KNUDSON, of Cornell University. KNUDSON has shown that the orchid seedlings apparently begin life as saprophytes, dependent upon the fungus to hydrolyze the starch of the natural or the cultural substrate into glucose. Seeds provided with nutrient salts alone, or with salts plus starch, will not germinate, but if provided with the proper fungus on the starch, or with a glucose substrate and no fungus, they will thrive perfectly well. The glucose relation seems to be an obligate one, for a similar molecular concentration of other sugars fails to promote growth. The fungus relation, though obligate at first in the absence of glucose, is unnecessary later; for sterile sugar-fed seedlings were later transferred to sugarless uninoculated media and continued to grow. The symbiosis is not specific, for germination was induced by fungi other than the true orchid fungus. The fungus, a friend at first, is apparently at bottom an enemy; for it acts as a pathogen if the food supply is low, and may even prevent germination altogether. Pathogenicity seems to be specific in some instances, for fungus isolated from *Cattleya* and *Epipactis* killed seed of *Odontoglossum*.

There is no evidence yet presented that the fungus is of value unless the constancy of the association of fungus and orchid be assumed as evidence of a significant relationship. The inherent characteristics of the seed which make germination slow and uncertain must be ascribed to the lack of reserve foods and the purely saprophytic character of the embryos during the critical periods of growth.

The last two papers were presented by Dr. WALTER T. SWINGLE, of the U. S. Department of Agriculture, with ROY W. NIXON as joint author in

one of them. According to Mr. SWINGLE, experiments that have been under way for the past three years at the U. S. Date Experiment Station at Indio, California, and at other points in California and Arizona, have demonstrated very strikingly the influence of pollen, not only on the size, shape and character of the seed, but also on the size, shape and character of the fruit of the date palm, and what is still more striking, on the time of ripening. It seems impossible to reconcile the observed effects of different kinds of date pollen with the existing theory of the influence of the male gamete. The double fertilization of the egg cell and the endosperm mother cell explains satisfactorily the influence of the pollen on the date seed itself, this being a form of xenia. However, the striking influence on the size, shape, character, and time of ripening of fruit cannot be explained by the theory of xenia, but apparently necessitates the recognition of influences in the plant body analogous to those exerted by ductless glands in the animal body. The simplest hypothesis is that the embryo, or the endosperm of the date seed, or both together, constitute a ductless gland apparatus that affects, by secreting hormones, the whole development of the date fruit, including tissues belonging morphologically to the mother plant, and especially to the thickened ovary walls which constitute the edible portion of the date fruit.

Similar effects have been noted also in citrus fruits. The phenomenon is called meta-xenia.

In addition to the sectional and joint programs, there were two lectures of general interest by prominent physiologists. On Wednesday afternoon Prof. F. E. LLOYD, president of the American Society of Plant Physiologists, presented his wonderful moving picture account of the contractile vacuole of *Spirogyra*. And one of the two general evening programs was devoted to a lecture by Prof. F. A. F. C. WENT, of Utrecht, on plant movement. He discussed particularly the work done in his laboratory on the physiology of phototropism. He and his son have uncovered convincing evidence for the existence of a photolabile growth-controlling substance in dark-grown seedlings. Severed tips of *Avena* coleoptiles, offset to opposite sides of their decapitated stocks after irradiation, induced a bending *away* from the light, and always in the direction determined by the contact of the severed tip. Similar tips placed on decapitated plants of other species, as for instance stimulated oats tips transferred to unstimulated barley stocks, produced like results. And finally, the same results followed the application of blocks of gelatin on which the stimulated tips have been allowed to stand for a time, and into which the hypothetical growth-controlling substance had presumably diffused.

But the interest of plant physiologists was not limited to what went on within the four walls of their own auditorium, or the rooms in which they

held their round tables and joint meetings with other sections. There were a number of papers in other sections that now and again lured them afield. Space limitations prohibit discussion of all of these, but mention of a few may well find a place.

Dr. WILLIAM SEIFRIZ, of the University of Pennsylvania, presented some of the results of his work on the structure of protoplasm. By means of magnetic fields applied to bits of nickel and cobalt introduced into cells by means of most ingenious and skilful micromanipulations, he has been able to gain some new information on the elasticity of their living contents. His results lead him to hold for a fibrous structure of protoplasm, as against the alveolar and other theories.

Prof. W. SZAFER, of the University of Krakau, presented a new successional series from the peat bogs of Poland, giving evidence of the climate of that part of Europe during the last interglacial period. His paper was supplemented in a way by Prof. H. C. COWLES, who discussed the succession point of view in floristics. The whole succession program of the section for ecology, participated in by W. H. PEARSALL, W. S. COOPER, G. E. DURIEZ and A. G. TANSLEY, held many points of interest for physiologists, as did also some of the forestry papers, notably those on root habits of tree seedlings, and the influence of light and shade. Mention should also be made of the gene symposium on Thursday night, participated in by R. A. EMERSON, E. M. EAST, HERMAN J. MULLER and WILLIAM H. EYSTER, who presented the gene as the basis of life, and controller of variation and heredity.

A part of the discussions in the sections for bacteriology, mycology, and pathology were to be devoted to the questions of the validity of taxonomic groupings based on physiological specificities where no morphological differences are evident. In tangled questions of this sort, obviously no definite conclusions can be expected at once; but it was felt that progress had been made toward a mutual understanding, and that satisfactory legislation on the subject may some day be looked for.

On Friday afternoon the physiologists met with the bacteriologists to hear a discussion of oxidation-reduction potentials and their measurement, by WILLIAM MANSFIELD CLARK, of the U. S. Hygienic Laboratory, Washington. It was a technical, and incisively critical discussion of the previous work on measurements of these potentials in biological materials. There is, however, some hope that indicators may be found which will make such measurements as practical as measurements of hydrogen ion concentration. Most of the past work is entirely untrustworthy according to Dr. CLARK, and will have to be repeated with more accurate methods when they have been developed.

Physiologists are usually willing to admit that discussions in sections for systematic botany go completely over their heads; but at Ithaca there were several things in the taxonomy section very much worth hearing, especially the papers on plant distribution. Notable among these were those of A. W. HILL, of Kew, on the Antarctica problem, of Prof. M. L. FERNALD, of Harvard University, on the post glacial flora of the northern hemisphere, and of Dr. C. H. OSTENFELD on the flora of the contemporary glacial period in Greenland.

A report of the Congress would be incomplete without mention of the social features, and general arrangements for the meeting. The physiologists held their dinner Wednesday evening in Prudence Risley Hall, and it was well attended. After dinner speaking was taboo, and it was made a time of informal good fellowship.

The general arrangements were almost ideal, thanks to the excellent work of the local committee on arrangements, and the program committee. Especially commendable were the efforts of Drs. L. W. SHARP, L. C. PETRY and H. H. WHETZEL, who seemed to be everywhere at once, overseeing everything and overlooking nothing that contributed to the comfort of attendants or to the success of the Congress. The registration went off smoothly and without crowding, the visitors were all quickly and satisfactorily quartered, and the ample spaces of Willard Straight Hall afforded abundant opportunity for the "visiting around" that is the real heart of a scientific gathering, as well as for the more ambitious but very comfortable social events that were staged. The programs were ready on the opening day, and abstracts in mimeograph form were distributed as soon as the sections began their sessions. The auditoriums were adequate, and in convenient proximity to each other, and the projection lanterns were in working order almost without exception. It was the general feeling of those who attended the Congress that it had been the best arranged and most profitable botanical meeting ever held in the United States. As an example of smooth functioning, the meeting was a joy, not only to the physiologists, but to all who were fortunate enough to be in attendance.

At the close of the Congress it was decided to hold the next International Botanical Congress at London, England, in 1930. Every one should plan if possible to attend the Fifth International Botanical Congress at that time.

TEMPERATURE EFFECTS IN THE METABOLISM OF WHEAT*

W. E. TOTTINGHAM¹

(WITH THREE FIGURES)

Introduction

Early observations of environmental effects in plant growth disclosed certain relations between temperature and rate of elongation. In recent decades attention has been turned to the influence of temperature upon the rates of specific reactions in the organism and upon the gross composition of plant tissues. Behind such investigations has been recognition that modifications of metabolism and composition may be of special significance in affecting food quality, disease resistance and fruitfulness. Development of technique for determining the specific effects upon plant metabolism of individual climatic factors is relatively recent. As examples, one may refer to the climatic chambers of HOTTES (8), the Wisconsin soil temperature tanks (9) and the installation of controlled greenhouses at the Boyce-Thompson Institute (3). The writer (17) has previously published results in this field.

Two main courses of procedure become immediately apparent in approaching the problem of temperature effects in metabolism. On the one hand, one may examine the composition of organs and tissues, on the other, he may compare the rate of reaction in isolated systems, such as specific enzymatic changes. In either event, limiting values of other environmental factors than temperature must be avoided, as pointed out by BLACKMAN (1). In view of the lack of adequately controlled experiments in which compositional responses of the plant to temperature have been determined, we have proceeded on this course, with the recognition, however, that the two courses are mutually dependent and complementary.

Apparatus and methods

CLIMATIC CHAMBERS

For use in the present work the culture chambers were much improved from their earlier form. They were identically exposed to sunlight in

* These investigations were supported by grants from the University research funds. Published with permission of the Director of the Wisconsin Agricultural Experiment Station.

¹ With the assistance of E. J. RANKIN, A. D. DICKSON and H. W. LOUWSMA. Credit is due certain of the writer's colleagues for advice and assistance freely given, especially to Dr. J. G. DICKSON for aid in operating the chambers, and ERIC MILLER for access to special records of the U. S. Weather Bureau.

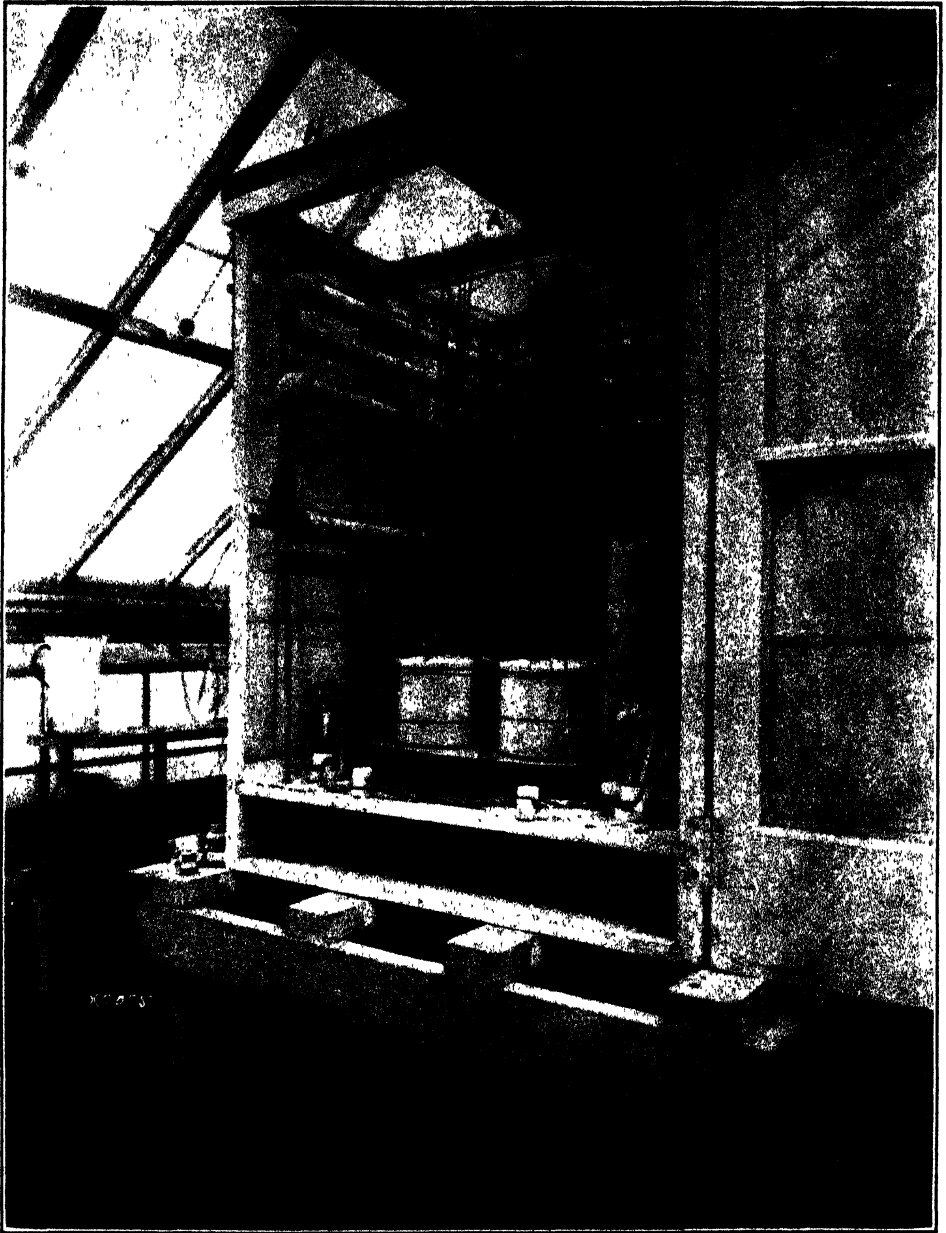


FIG. 1. Arrangement of culture chambers used. A. Illuminating lamps. B. Sockets of cylindrical heaters. C. Thermostats and humidostats, shaded. D. Hygrothermograph. E. Rotating table. F. Location of humidifier, door removed. G. Location of blower. H. Water cell.

separate compartments of a greenhouse range and attached to refrigeration service. One of these compartments was regulated roughly to a temperature of about 20° C, the other about 24° C. Chilling was effected by about 9.0 m. (30 ft.) of 3.2 cm. (1.25 in.) brine pipe supported in coils on the sides of the upper part of the chamber. By means of valves and by-passes the effective brine flow was reduced to about 3.0 m. at the higher temperature. Heaters in the form of luminous radiator units of 250 watts capacity each, erected on the floor of the culture chamber at the corners, operated against the brine coils. These functioned intermittently under the control of a bimetallic thermostat suspended at the top-center of the chamber.

The humidifying system was altered by removing the toweling, transferring the exit to the end of the tank and erecting adjacent thereto a spray nozzle which played into the approaching air current. Separate lines of hot and cold water were merged to give some control over the temperature of the mist delivered. To conserve space the humidifier was placed beneath the chamber with its exit connected upward thereto. At its entrance was fixed an electrically operated blower.² At first the latter was operated intermittently through a humidostat, but this proved too much of a tax upon the motor. In later tests the blower has operated continuously while its service is cut in or out by a damper. The latter is closed by a solenoid operated through the humidostat.³ This latter control instrument⁴ is a wooden cylinder so suspended as to effect a contact after the manner of a thermostat, and sensitive to small changes in relative humidity.

Previously experienced difficulty in warping of the rotating tables, which bear the plant cultures, was avoided by constructing them wholly from iron. The general arrangements of the culture chambers are to be seen in fig. 1, while the degree of control over temperature and humidity appears in fig. 2.

ILLUMINATION

In the particular study here attempted it is obviously important that illumination be kept above the plane of a limiting factor in growth. In this respect the results should be subjected to critical valuation.

Inasmuch as tests early showed the loss of light from the north side of the culture chambers this sash was closed by a sheet of asbestos. Shades were omitted from the greenhouse roof, but muslin curtains were fixed on the vertical sash of the chambers, so as to slide horizontally. The usual

² Sirocco no. 0 of the American Blower Company, Detroit.

³ Acknowledgment is due the General Electric Company for assistance in the installation of magnetic switches and solenoids adapted to this apparatus.

⁴ Supplied by the Johnson Service Company, Milwaukee, Wis.

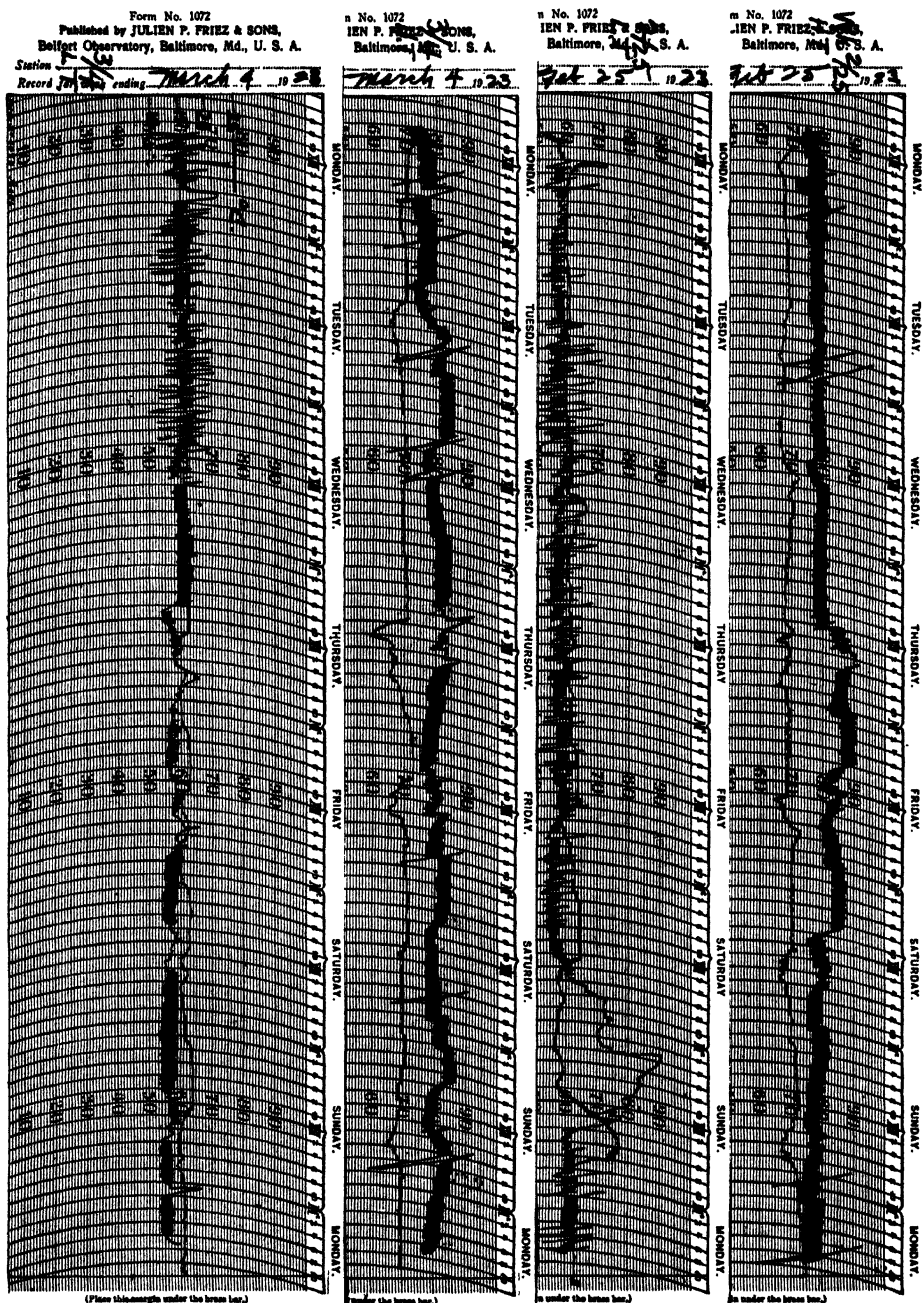


FIG. 2. Examples of control of temperature and humidity in the culture chambers.

glazing applied to the greenhouse roof has been omitted also, in the more recent tests. The artificial illumination was increased in successive tests from one 500 watt Mazda lamp to three lamps of a total capacity of 2,500 watts, which consumed the maximum current available for each chamber. In order to derive maximum illumination, by dropping the lamps close to the chambers, it was necessary to provide for elimination of excessive heat radiation. For this purpose shallow copper trays were placed upon the chambers,⁵ after removing the upper panes of the top sash. These were of the same dimensions as the chamber top and bore panes of glass overlying those of the sash. The necessary plumbing attachments provided for a sheet of water flowing across this cell, the maximum depth of water being set at about 1.0 cm. With this attachment, satisfactory control over temperature was maintained except during sunny middays of early fall and late spring.

With the use of daylight it is obvious that both quality and intensity of illumination varied widely. However, these variations must have been closely parallel in the two culture chambers. It is possible to state the approximate intensities effective. Using a Sharp-Millar photometer, with a selective filter solution of both chromate and di-chromate of potassium, the intensity in the shaded chambers on a cloudy day was found to be about 0.2 that out of doors. The out-door values of sunlight have been derived from the pyr heliometric measurements of the local station of the U. S. Weather Bureau, made with a Callendar recorder. In converting these to foot-candle equivalents, data of KIMBALL have been employed. He compared measurements by a carefully standardized Sharp-Millar photometer with the simultaneous records of the pyr heliometer at Mt. Weather, Virginia, late in January. These data give an average equivalent of 139 foot candles for 1 calorie per sq. cm. per hour. Other comparisons of his photometric measurements on several dates from mid-October to mid-May, the period of operation of our chambers, with the corresponding pyr heliometric values show considerable variability in the course of seasons, but with an average of 145 foot candles per hour-calorie. We have adopted the conservative value of 140 foot candles as equivalent to 1 hour-calorie of solar radiation. Assuming the same proportion of illuminating value in the radiation admitted by the chambers, the intensity of the latter would be 28 foot candles per hour-calorie of solar radiation out of doors. Direct measurements of illumination delivered by the lamps overhead were made with the Sharp-Millar instrument. The mean hourly maximum radiation has been derived from the corresponding values in records of the local station of the Weather Bureau, making adjustment for the proportions of monthly periods involved in each culture series.

⁵ Upon the advice of Dr. M. LUCKIESCH, Nela Research Laboratories.

On the basis of the relations just stated, the illumination effects in our chambers appear to have been about as follows: Efficiency of reflectors, 150 per cent.; maximum illumination from reflectored 1,000 watt lamp at soil surface 150 foot candles, at half depth of chamber 350 foot candles; range of mean maximal solar radiation, 590 foot candles in December to 1,800 in May,⁶ with shades closed. Depending upon the degree of artificial illumination, this would place the mean maximal total illumination of the upper leaf surface of wheat when half grown at about 750 to 1,950 foot candles in our earliest tests and 1,450 to 2,650 in the latest ones, with the chambers shaded. In the later tests more sunlight was admitted to the chambers, at some expense of temperature control, and the mean maximal light intensities must have been considerably higher than those just stated.

There are small grounds for comparing these values with plant requirements. More accurate measurements of light intensity and determinations of the demand at different growth phases are much needed. According to HENDRICKS and HARVEY (6), wheat grown at 14° C. under continuous illumination by Mazda lamps requires 185 to 225 foot candles for seed production, while buckwheat at 25° C. requires about one half this intensity. It appears possible that the requirements in this respect may vary with the stage of growth. However, if we assume that the minimum intensity required varied inversely as the time of exposure, the results of HENDRICKS and HARVEY indicate a light requirement of wheat approaching 450 foot candles for a 12 hour day. This would correspond to a diurnal maximum of about 845 foot candles. If one neglects the matter of light quality, it appears that our earlier wheat cultures suffered from insufficient illumination. The adequacy of the light factor will be treated more fully in the general discussion.

NUTRIENT SOLUTION

To provide for separate variations in supply of some of the more essential nutrient elements the formulae of table I were used.

This solution provides for the relatively high requirements of young wheat plants for both potassium and nitrogen (5) and also permits separate varying of the supply of these elements. The use of sodium chloride throughout compensates somewhat the reduction in concentration of sodium and chlorine as the contents of nitrogen and potassium are altered. This formula is designated by the term "full." With the supply of the NaNO_3 reduced to one half and one fifth the full amount, the designations "0.5N" and "0.2N," respectively, are applied. The corresponding variations of potassium are designated as "0.5K" and "0.2K."

⁶ The actual maxima exceeded these values by about 600 foot candles.

TABLE I

FORMULAE OF SALTS FOR A SOLUTION PERMITTING SEPARATE VARYING OF THE POTASSIUM AND NITRATE CARRIERS

FORMULAE OF SALTS USED	CONCENTRATION OF ANHYDROUS SALTS
	Per cent.
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.025
$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	0.040
NaCl	0.025
KCl	0.075
NaNO_3	0.075
Ferric citrate	0.001

Cultural treatment and results

Throughout the present work pure line seed of the Marquis variety of wheat has been used, selected for uniformity of size from stock not over two years old. Unless stated otherwise, temperature control began with the germination phase of growth. Details of the procedure for water culture will be found elsewhere (16). The climatic records are given for the period of simultaneous growth at the two temperature ranges in each test. These were taken in part on hygro-thermographs, but the adjustments for humidity control were directed toward equalizing the evaporation from atmometers carried on the rotating tables. Unless specified otherwise, the plants were killed promptly after harvesting by heating about 30 minutes at 98° C. in a closed oven. Subsequent desiccation occurred at about 70° C. with ventilation. The roots were severed from the tops to prevent translocation of materials during drying.

WATER CULTURES

SERIES 1.

Seedlings selected from a large group were set up in triplicate cultures of five plants each for each variation of the cultural treatment. At the higher temperature this transfer occurred seven days after placing the soaked seeds upon the germination net. These seedlings were then about 10 cm. tall and three times as tall as those grown at the lower temperature, which were set up two days later. The culture vessels had a capacity of 960 cc. (quart Mason jars) and the solutions were renewed twice a week. Throughout the test the full concentration of nutrients was employed but during germination the P_H was adjusted to about 7. At the higher temperature the roots early developed an abnormal mucous coating, while the

advent of withering in the tops of these cultures marked the termination of the test. As a result of this experience the maximal temperature in later series was advantageously reduced.

This series grew from December 1, 1922, to January 10, 1923, including the germination periods in the respective chambers. The following climatic conditions prevailed in the chambers: Temperature about 25° and 15° C.; relative humidities about 70 and 50 per cent.; maximum artificial radiation 175 foot candles; total solar radiation 970 calories; mean hourly maximum of solar radiation 650 foot candles; mean hourly solar radiation (eight hour day) 410 foot candles; evaporation from the standard black atmometer 559 cc. at the higher temperature, and 517 cc. at the lower, or an excess of 8 per cent. at the higher temperature.

Linear measurements of the plants were rather uniform within each temperature group, but the tops were about 40 per cent. taller at the lower temperature. The yield and composition of tops are given in table II. In

TABLE II

COMPOSITION OF DRY TOPS OF SERIES 1. GROWTH PERIOD 41 DAYS. AVERAGE TOTAL ILLUMINATION IN 8-HOUR DAY, 580 FOOT CANDLES

TEMPER- ATURE	NUTRIENT SUPPLY	YIELD OF DRY MATTER ^a	ETHER EXTRACT	POLYSAC- CHARIDES ^b	CRUDE PROTEIN ^c	YIELD OF CRUDE PROTEIN
° C.		Ggm.	Per cent.	Per cent.	Per cent.	mg.
25	Full	54	6.6	7.1	7.1	44
25	0.5N	54	8.7	9.7	6.9	37
25	0.2N	54	11.4	8.9	6.9	37
25	0.5K	54	7.1	5.2	7.1	38
25	0.2K	48	7.0	9.1	7.1	34
15	Full	84	11.5	12.1	7.1	60
15	0.5N	87	10.2	10.3	5.7	50
15	0.2N	78	10.7	9.8	5.7	44
15	0.5K	81	10.5	12.3	7.8	63
15	0.2K	84	9.6	10.0	5.8	49

^a Total of 3 cultures, or 15 plants.

^b Hydrolysis of sugar-free tissue for 1 hour with 3.7 per cent. H_2SO_4 .

^c N \times 6.25 in residue from acid hydrolysis.

view of the limited growth these merit little discussion. It appears probable that the illumination was considerably deficient. The yield was greater at the lower temperature by 60 per cent. Likewise, the contents of ether extract and polysaccharides were generally favored by the lower temperature.

SERIES 2.

Twenty-five seedlings left by selection from about 200 were transferred on each germination net to a battery jar of 3.5 liters capacity. This was coated with asphaltum paint to exclude light. The plane of nutrients was increased by steps from 0.2 full to full concentration, while a P_H value of about 7 was maintained throughout the test. In the final week the solutions were not renewed, so as to accentuate the relative absorption of nutrients. Drying of samples for analysis was here conducted throughout at about 55° C. As a special phase of the analytical examination of the plant tissues, determinations were made of the proportion of pentoses present, by fermentation of the hexoses with a pure strain of yeast.⁷

This series grew from February 18 to March 30, 1923, being terminated only by limitation of time. The following climatic conditions prevailed: Temperature planes about 23° C. and 13° C., with relative humidities of about 80 per cent. and 60 per cent. respectively; maximum artificial radiation at mid-chamber 175 foot candles; total solar radiation 3,040 calories; mean hourly maximum of solar radiation 1,310 foot candles; mean hourly solar radiation (basis of 10 hour day) 1,040 foot candles; total evaporation from the standard black atmometer 616 cc. at the higher temperature and 516 cc. at the lower or 19 per cent. greater in the former case. Solar radiation effective during the test of absorption of nutrients averaged 95 calories per day, equivalent to a maximum of about 2,000 foot candles, and an average total illumination of 10 hour day, 1,500 foot candles (1,720 foot candles on day of harvesting).

The only significant variation in linear measurements was an average elongation of the tops at the higher temperature of about 40 per cent. Both tops and roots were considerably greater in diameter at the lower temperature. Tables III, IV and V show the yield, composition and absorption values.

With due allowance for difference in numbers of plants involved, the production of dry matter was much more extensive in this series than in the previous one. This difference is greater than can be accounted for by the difference in intensity of solar radiation for the growth periods. The yield and composition of tops were much the same for the two planes of temperature. Only the insoluble protein was consistently higher in the plants grown at the lower temperature. The pentose content of the hemicellulose was somewhat lower also in this case. The roots were a little heavier at the lower temperature than the higher one and contained, as an average, over 50 per cent. more "available" carbohydrates in the former case. On a reduced supply of nitrate, protein synthesis appears to have been hindered

⁷ Under the direction of Dr. E. B. FRED, bacteriologist of this institution.

TABLE III
COMPOSITION OF DRY TOPS OF SERIES 2. GROWTH PERIOD, 41 DAYS. AVERAGE TOTAL ILLUMINATION OF 10-HOUR DAY, 1,210
FOOT CANDLES. DRY WEIGHT BASIS

TEMPER- ATURE	NUTRIENT SUPPLY	YIELD OF DRY MATTER	ETHER EXTRACT	DEXTRINS AND STARCH	HEMICEL- LULOSE ^a	PENTOSE OF HEMICEL- LULOSE	INSOLUBLE PROTEIN	YIELD OF INSOLU- BLE PRO- TEIN
° C.		Gm.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	mg.
23	Full	3.01	5.4	1.0	13.6	9.9	4.6	138
23	0.5N	3.33	6.7	1.0	13.7	9.9	4.5	150
23	0.2N	2.98	3.5	1.0	14.0	9.2	4.3	128
23	0.5K	3.63	6.1	0.8	13.8	8.5	4.5	163
23	0.2K	3.74	6.8	0.8	13.9	9.3	4.5	168
13	Full	2.71	7.2	0.6	9.3	6.4	6.2	162
13	0.5N	3.09	6.6	1.2	12.0	7.2	6.9	213
13	0.2N	3.17	6.8	1.3	12.9	7.2	5.6	177
13	0.5K	2.79	6.0	0.8	12.1	5.6	8.1	226
13	0.2K	2.81	7.3	0.7	12.9	8.4	6.3	177

^a Hydrolysis for 1.0 hour with 2 per cent. H₂SO₄.

at the higher temperature and favored at the lower, but the samples were too small to give very significant results.

TABLE IV

COMPOSITION OF DRY ROOTS OF SERIES 2. GROWTH PERIOD 41 DAYS. AVERAGE TOTAL ILLUMINATION OF 10-HOUR DAY, 1,210 FOOT CANDLES. DRY WEIGHT BASIS

TEMPERATURE	NUTRIENT SUPPLY	YIELD OF DRY MATTER	SUGARS AND POLYSACCHARIDES ^a	INSOLUBLE PROTEIN	YIELD OF INSOLUBLE PROTEIN
° C.		Cgm.	Per cent.	Per cent.	mg.
23	Full	39	14.4	7.8	31
23	0.5N	41	13.1	6.4	26
23	0.2N	56	10.8	5.7	32
23	0.5K	42	11.6	7.4	31
23	0.2K	39	13.7	7.6	30
13	Full	56	20.1	6.8	38
13	0.5N	63	18.8	8.1	51
13	0.2N	54	19.2	8.3	45
13	0.5K	53	19.5	6.2	33
13	0.2K	49	20.1	6.0	29

^a Ether extract not removed. Hydrolyzed 1 hour with 2 per cent. H_2SO_4 .

The data for absorption of nutrients show increased up-take of potassium at the higher temperature and of nitrogen at the lower, on the full solution. At the lower planes of supply of potassium the results indicate a greater requirement of this element at the lower temperature. This is in accord with the results of GERICKE (4). An increased requirement for nitrogen at the higher temperature is indicated by the increased absorption of this element on the partial planes of supply. With the lowest supply of the

TABLE V

ABSORPTION FROM NUTRIENT SOLUTION OF SERIES 2. AVERAGE TOTAL ILLUMINATION OF TEST PERIOD IN 10-HOUR DAY, 1,500 FOOT CANDLES. EVAPORATION FROM STANDARD ATMOMETER, 57.8 CC. AT 23° C., AND 57.6 CC. AT 13° C.

ELEMENT TESTED	TEMPERATURE °C.	NUTRIENT SUPPLY				
		Full	0.5N	0.2N	0.5K	0.2K
Nitrogen	23	10 ^a	56	-31 ^b	8	10
Nitrogen	13	19	37	40	12	18
Potassium	23	32	21	26	15	42
Potassium	13	19	16	26	20	61

^a Percentage of the amount presented.

^b Recovery of 131 per cent.

element the results give evidence of a return to the last portion of solution of unassimilated nitrate, which had been absorbed from previous supplies. This negative balance of nitrogen might be expected to eventuate critically if prolonged, but the data of yields and composition give no distinct evidence of such conditions. It is possible that cloudiness of the day preceding harvesting, when an average total radiation of about 1,100 foot candles was effective, induced a change of energy relations in the plant which were responsible for the excretion of nitrate. HOAGLAND (7) has shown the importance of illumination in this regard. While much more adequate than in series 1, the illumination may have been somewhat deficient here.

SERIES 3.

This test was conducted in the same manner as the previous one, excepting the increase of plants per culture to 40. Development of seedling blight (*Helminthosporium*) occurred to some extent at the higher temperature. The seriously affected plants were eliminated in preparing the tissue for chemical analysis.

Growth here covered the period from October 24 to December 24, 1923. The climatic conditions effective in the chambers were as follows: Temperature planes, about 23° and 13° C.; maximal artificial radiation at mid-chamber, about 340 foot candles; total solar radiation 1,590 calories; mean hourly maximum of solar radiation, 670 foot candles; mean hourly solar radiation (basis of 8-hour day) 450 foot candles; evaporation from the standard black atmometer 524 cc. at the higher temperature and 556 cc. at the lower, or about 6 per cent. excess in the latter case. Solar radiation effective during the measured absorption of nitrate averaged 17 calories

TABLE VI

YIELD AND ABSORPTION OF NUTRIENTS IN SERIES 3. GROWTH PERIOD, 62 DAYS. AVERAGE TOTAL ILLUMINATION OF 8-HOUR DAY, 790 FOOT CANDLES

NUTRIENT SUPPLY	TEMPERATURE	DRY MATTER OF TOPS	DRY MATTER OF ROOTS	ABSORPTION OF NITRATE
	° C.	Ggm.	Ggm.	Per cent.
Full	23	73 ± 0	27 ± 2	16.8 ± 0.4
Full	13	95 ± 6	32 ± 1	14.1 ± 0.2
0.5N	23	64 ± 6	25 ± 3	- 32.7 ± 4.5*
0.5N	13	90 ± 6	31 ± 1	- 38.4 ± 2.7
0.2N	23	70 ± 4	26 ± 1	51.1 ± 2.1
0.2N	13	91 ± 0	38 ± 1	43.7 ± 10.6

* Negative absorption denotes increased concentration in the solution by leaching from the plants.

per day, equivalent to a maximum of about 360 foot candles, and average total illumination of 8-hour day, 630 foot candles (950 foot candles on day of harvesting).

TABLE VII
COMPOSITION OF DRY TOPS IN SERIES 3

NUTRIENT SUPPLY	TEMPERATURE	ETHER EXTRACT	REDUCING SUGARS	POLYSACCHARIDES ^a	INSOLUBLE PROTEINS ^b	YIELD OF INSOLUBLE PROTEIN
	° C.	Per cent.	Per cent.	Per cent.	Per cent.	mg.
Full	23	6.8 ± 1.0	1.6 ± 0.2	12.8 ± 1.7	2.9 ± 0.4	21
Full	13	8.1 ± 1.0	0.5 ± 0.1	7.3 ± 0.3	5.2 ± 0.9	49
0.5N	23	9.2 ± 1.8	1.1 ± 0.9	8.0 ± 0.9	3.5 ± 0.2	22
0.5N	13	9.1 ± 1.8	0.4 ± 0.1	7.2 ± 0.4	10.7 ± 0.0	96
0.2N	23	6.5 ± 0.3	0.5 ± 0.0	3.7 ± 1.6	3.3 ± 0.1	23
0.2N	13	9.4 ± 0.5	0.3 ± 0.0	3.6 ± 0.1	9.3 ± 0.3	85

^a Hydrolysis 1 hour with 2 per cent. H_2SO_4 .

^b N × 6.25 in residue from acid hydrolysis.

The results of this series appear in tables VI, VII and VIII. Both tops and roots gave an average increase of about 30 per cent. in dry weight at the lower temperature. The only differences in chemical composition which appear significant occurred in the tops. Here the polysaccharide content was favored by the higher temperature on the full nutrient solution, while insoluble protein was most abundant throughout at the lower temperature. Reducing sugars were appreciably more plentiful in the tops at the higher

TABLE VIII
COMPOSITION OF DRY ROOTS OF SERIES 3

NUTRIENT SUPPLY	TEMPERATURE	ETHER EXTRACT	REDUCING SUGARS	POLYSACCHARIDES ^a	INSOLUBLE ^b PROTEIN	YIELD OF INSOLUBLE PROTEIN
	° C.	Per cent.	Per cent.	Per cent.	Per cent.	mg.
Full	23	6.8	0.5	21.4	5.1	14
Full	13	4.7	0.5	19.3	3.4	11
0.5N	23	5.4	0.5	20.7	3.7	9
0.5N	13	4.8	0.5	26.8	2.7	8
0.2N	23	5.0	0.5	20.3	4.7	12
0.2N	13	4.4	0.6	19.2	2.9	11

^a Hydrolysis 1 hour with 2 per cent. H_2SO_4 .

^b N × 6.25 in residue from acid hydrolysis.

temperature and the same applies to insoluble protein in the roots. A negative balance of nitrate occurred in all cultures at the 0.5N concentration. It appears most probable that the difference from series 2, where this effect was found in the 0.2N solution at the higher temperature, should be ascribed to the differences in illumination. Apparently the plant is quite sensitive to this factor in the aerial environment. The average light intensity of this test was much less adequate than in the preceding one and obviously deficient, as indicated by the yields.

SAND CULTURES

SERIES 4.

Sand of high purity was used,^a composed of spherical particles grading 60 to 70 mesh. Portions of 3.2 kgm. each were used in stoneware jars of 1.9 liters (2 quart) capacity. The water plane was increased by three steps from 25 per cent. of the holding capacity at planting to 50 per cent. at active growth, and reduced later to favor maturation of the plants. Nutrient salts were added in solution in three successive increasing portions. The total amounts per culture for the full solution were as follows: NaNO_3 1.0 gram, equi-molar mixture of $\text{Na}_2\text{HPO}_4 \cdot 10\text{H}_2\text{O}$ and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 0.15 gram, KCl 0.2 gram, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 0.1 gram, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 gram and ferric citrate 0.04 gram. Surface sterilization for the suppression of seedling blight (*Helminthosporium*) was effected by the use of semesan, an organic mercurial preparation. Fourteen seedlings per culture were left

TABLE IX

YIELD AND COMPOSITION OF STRAW IN SERIES 4. GROWTH PERIOD, 80 DAYS. AVERAGE TOTAL ILLUMINATION OF 12-HOUR DAY, 1,150 FOOT CANDLES. DRY WEIGHT BASIS

NUTRIENT SUPPLY	TEMPERATURE	YIELD OF DRY MATTER	NITRATE RADICLE	SOLUBLE PROTEIN ^a	INSOLUBLE PROTEIN ^b	YIELD OF INSOLUBLE PROTEIN
	° C.	Gm.	Per cent.	Per cent.	Per cent.	mg.
Full	21-22	1.43 ± 0.33	2.3	4.4	8.7	124
Full	16-17	4.63 ± 0.23	0.5	3.8	8.7	403
0.5N	21-22	1.73 ± 0.13	1.1	5.6	8.1	149
0.5N	16-17	4.00 ± 0.10	0.2	3.1	8.1	344
0.2N	21-22	1.38 ± 0.03	0.6	3.1	8.7	120
0.2N	16-17	3.30 ± 0.25	0.2	1.9	6.3	208

^a Non-nitrate N × 6.25 in water extract.

^b N × 6.25 in residue from watery extraction.

^c Supplied by Ottawa Silica Company, Ottawa, Illinois.

by selection from three times that number. After two months of growth the plants subjected to the lower temperature were equal in height to contemporaneous greenhouse cultures on soil, while those at the higher temperature were considerably shorter and characteristically spindling. Despite hand pollination from plants blooming in the greenhouse only two or three shriveled seeds were produced, and these at the higher temperature. This result may have been due to unfavorable effects of the temperatures upon pollen growth, as well as to cumulative effects upon the plant as a whole. Heads are included in the straw weights but they were discarded in preparing the tissue for analysis. The analytical data appear in table IX.

Growth after emergence from the soil occurred from March 4 to May 19, 1924, in the warmer case and four days later in the colder one. The climatic data for this series were as follows: Temperature planes 16–17° C. and 21–22° C.; maximal artificial radiation at mid-chamber 340 foot candles; total solar radiation 5,320 calories; mean hourly maximum of solar radiation 1,300 foot candles; mean hourly solar radiation (basis of 12-hour day) 810 foot candles; evaporation from the standard black atmometer 615 cc. at the lower temperature and 672 cc. at the higher. Thus the evaporating power was about 9 per cent. greater at the higher temperature.

The yield was nearly 150 per cent. greater at the lower temperature and here also production increased with the nitrate supply. At the higher temperature the total nitrogen content of the tissue was higher, which is practically equivalent to stating that carbohydrates were most abundant at the lower temperature. It increased with the plane of supply, most distinctly at the lower temperature, the variations occurring in soluble forms. On the higher planes of supply the nitrate content of the tissue at the higher temperature was more than inversely proportional to yield, indicating a greater capacity for assimilating this radicle at the lower temperature.

SERIES 5.

This series of wheat cultures was conducted in the same manner as the previous one with the exception of a portion of the environmental exposure. When it appeared that the blossoms were formed and pollination was approaching, the cultures were gradually removed to a greenhouse exposure of about 20° C. until seeds were formed. They were then gradually subjected to the original temperatures of their respective chambers. This removal period extended from April 13 to April 29 at the higher temperature and April 28 to May 14 at the lower one, the former interval corresponding to 3,130 calories of solar radiation effective in the house, the latter to 3,750. In tables X and XI are assembled the data of yields and composition.

TABLE X

YIELD AND COMPOSITION OF GRAIN IN SERIES 5. GROWTH PERIOD, 99 DAYS. AVERAGE TOTAL ILLUMINATION OF 12-HOUR DAY, 1,560 FOOT CANDLES

NUTRIENT SUPPLY	TEMPERATURE	DRY MATTER	AVERAGE WEIGHT PER SEED	PROTEIN CONTENT ^a	TOTAL YIELD OF PROTEIN
	° C.	mg.	mg.	Per cent.	mg.
Full	18-20	87 ± 40	10.5 ± 1.5	27.5	48
Full	12-14	214 ± 112	13.5 ± 0.5	28.7	123
0.5N	18-20	130 ± 43	14.0 ± 2.0	24.4	63
0.5N	12-14	458 ± 108	16.5 ± 1.5	23.4	214
0.2N	18-20	65 ± 21	25.5 ± 3.5	19.3	25
0.2N	12-14	400 ± 123	18.5 ± 2.5	18.1	145

^a Total N × 6.25.

This series grew in the period February 18 to May 27, 1925, at the higher temperature, extending to June 10 at the lower one. The climatic data follow: Temperature ranges, 13-15° C. and 18-20° C.; relative humidity, about 55 and 75 per cent. respectively; maximum artificial radiation, about 500 foot candles; total solar radiation effective in house or chamber, 9,560 and 9,940 calories at the higher and lower temperatures respectively; mean hourly maximum of solar radiation, 1,740 and 1,810 foot candles respectively; mean hourly radiation of 12-hour day, at high temperature 900 foot candles in chamber, 2,280 in house, average 1,100; at low temperature 870 foot candles in chamber, 2,730 in house, average 1,140 foot candles; evaporation from the standard black atmometer 587 cc. at the lower temperature

TABLE XI

YIELD AND COMPOSITION OF STRAW IN SERIES 5

NUTRIENT SUPPLY	TEMPERATURE	DRY MATTER	CRUDE PROTEIN CONTENT ^a	CONTENT OF NITROGEN BASES ^b	TOTAL YIELD OF PROTEIN ^c
	° C.	Gm.	Per cent.	Per cent.	mg.
Full	18-20	2.41 ± 0.14	12.9	5.9	284
Full	12-14	4.44 ± 0.04	10.9	6.7	596
0.5N	18-20	2.32 ± 0.29	11.1	4.8	223
0.5N	12-14	3.69 ± 0.26	7.7	3.9	288
0.2N	18-20	1.44 ± 0.58	13.4	6.6	190
0.2N	12-14	3.20 ± 0.02	4.8	2.8	179

^a Total N × 6.25.

^b N precipitated by phosphotungstic acid × 6.25.

^c Dry matter × nitrogenous bases.

and 636 cc. at the higher one. Thus the evaporation was about 8 per cent. higher at the higher temperature.

It appears that the intensity of illumination could hardly have been seriously deficient for these cultures. The data of total yields show 60 to 120 per cent. increases in development of straw at the lower temperature and a corresponding increase of 150 to 500 per cent. in the grain. Despite these wide variations in growth the crude protein content of the grain differed little at the two temperatures. This seems not to hold for the straw. The plane of nitrate supply was more effective than temperature difference in affecting the content of protein of the seed. In the case of the straw, however, this response occurred only at the lower temperature.

Computation shows that 43 to 49 per cent. of the total nitrogen in straw of the high temperature cultures was precipitable by phosphotungstic acid, as compared with 51 to 62 per cent. at the lower one. The difference was most marked with the full nutrients, and is very likely due to inferior assimilation of nitrate at the higher temperature, as observed in series 4.

SOIL CULTURES

SERIES 6.

In order to correct still further the possible abnormal conditions of the environment, this series was grown on Miami silt loam to which a small application of complete fertilizer was added. Each 2-quart stoneware jar supported 15 plants. Optimal planes of soil moisture were maintained to maturity. The cultures progressed in a greenhouse, at temperatures ranging from 15° C. (60° F.) by night to 20° C. (70° F.) by day, until the seeds had formed. During the remainder of development they were divided into two lots, one lot being gradually adjusted to the conditions of each climatic chamber. The data of yields and composition appear in table XII.

TABLE XII

YIELD AND COMPOSITION OF SERIES 6. GROWTH PERIOD, 82 DAYS. AVERAGE TOTAL ILLUMINATION OF 13-HOUR DAY, 2,320 FOOT CANDLES

TEMPERATURE	YIELD OF DRY STRAW ^a	PROTEIN CONTENT OF STRAW ^b	YIELD OF DRY GRAIN ^a	AVERAGE WEIGHT PER SEED	PROTEIN CONTENT OF GRAIN ^a	AVERAGE YIELD OF PROTEIN
° C.	Gm.	Per cent.	Gm.	mg.	Per cent.	Cgm.
18-20	9.3 ± 1.3	3.1 ± 0.7	2.3 ± 1.0	11 ± 3	19.4 ± 5.0	73
12-14	10.4 ± 2.2	2.4 ± 0.1	3.5 ± 1.9	15 ± 6	15.6 ± 1.6	80

^a Average of 6 cultures ± average maximum departure.

^b Average of 3 cultures ± average maximum departure, determined by precipitation with phosphotungstic acid.

The total growth period after germination extended from March 24 to June 13, 1925, at the higher temperature and to June 17 in the other case, with the transfer to the chambers occurring on May 27. Climatic records for the entire growth period follow: Temperature ranges 13–15° C. and 18–20° C.; maximum artificial radiation about 500 foot candles; total solar radiation, 14,740 calories in the house and 2,010 calories in the chambers; mean hourly maximum of solar radiation, 3,490 foot candles; mean hourly solar radiation, 2,480 foot candles in house (basis of 13-hour day), 1,120 foot candles in chamber (basis of 14-hour day); evaporation during the control period from the standard white atmometer, 126 cc. at the higher temperature, and 116 cc. at the lower one. Thus the evaporation was about 9 per cent. greater at the higher temperature.

The yields of grain at the lower temperature averaged 35 per cent. greater than at the higher one, while there was a lesser difference of 10 per cent. in the case of the straw. This temperature effect is more marked if one eliminates the values of one immature culture at the lower temperature. The weight of individual seeds averaged 36 per cent. higher at the lower temperature. As would be expected, this was associated with increased starchiness at the lower temperature. In terms of the protein content the seeds produced at the higher temperature were richer by 4 per cent., based upon the dry matter. The seeds reared at the lower temperature had the same protein content as the grain sown. Based upon this value, the protein content increased 24 per cent. at the higher temperature. A similar increase occurred in the protein content of the straw.

SOIL CULTURES (IN CLIMATIC HOUSES)

SERIES 7.

After reaching the seed-filling stage in the general greenhouse under conditions similar to those of the previous series these cultures were divided into two groups. These were matured in the controlled houses which contained the culture chambers. They were transferred thereto on January 31. The humidity difference was not excessive in these two sections. A test in the closed houses gave vapor pressure deficits of 7 and 10 mm., with much closer values when outside air was admitted for temperature control. No atmometric records were taken. The data from the crops are given in table XIII.

The growth of this series covered the period December 1, 1925, to February 23, 1926. Climatic data were as follows: Temperatures, about 20° and 24° C.; artificial illumination, none; total solar radiation, 6,060 calories;⁹ mean hourly maximum solar radiation, 1,820 foot candles; mean

⁹ Based on photometric indications that the house value was 0.5 of outdoor illumination.

TABLE XIII

YIELD AND PROTEIN CONTENT OF SERIES 7. GROWTH PERIOD, 85 DAYS. AVERAGE SOLAR ILLUMINATION OF 8-HOUR DAY, 1,240 FOOT CANDLES

TEMPER- ATURE	NITRATE SUPPLY	YIELD OF SEED	PROTEIN CONTENT OF SEED	YIELD OF PROTEIN	YIELD OF STRAW	PROTEIN CONTENT OF STRAW	YIELD OF PROTEIN
° C.		Gm.	Per cent.	Cgm.	Gm.	Per cent.	Cgm.
16	Liberal	2.13	19.5 ^a	42	13.5	6.0 ^b	81
24	Liberal	2.04	23.5	48	12.2	5.0	61
16	Scanty	2.27	18.5	42	13.0	5.8	75
24	Scanty	1.79	24.5	44	12.5	4.9	61

^a Total N × 6.25.

^b Insoluble and coagulable N × 6.25.

hourly solar radiation, 1,240, based on an 8-hour day; evaporation estimated at not over 40 per cent. higher at the higher temperature than the lower one.

The results show clearly that temperature predominated over the supply of nitrate in determining yield and composition. At the lower temperature the yield of grain increased somewhat, while the protein percentage was considerably decreased. The yield of protein increased a little with the temperature. The yield of straw varied in the same manner as that of the seed, being favored by the lower temperature. In percentage and yield of protein, however, the organs responded quite differently. These functions were enhanced in the straw by the lower temperature. It appears that the production of protein in the straw is favored by the lower temperature, as the data indicate little difference in total transference of this constituent to the seed.

HUMIDITY VARIATION WITH SOIL CULTURES

SERIES 8.

In view of the rôle attributed by agronomists to aridity of climate in affecting the composition of grain, it seemed desirable to compare this factor with temperature. Soil cultures of wheat were brought to seed formation in the greenhouse on optimal planes of water. They were then matured with exposure to uniform illumination and temperature but different atmospheric humidity, in the climatic chambers. The illumination consisted of the maximal sunlight admissible without serious upset of temperature control, supplemented by all the artificial light available. During the 3 to 4 day interims between applications of water to the cultures, the soil moisture varied rather widely in response to the influence of

the atmospheric humidity, as in nature. The data of yield and composition appear in table XIV.

This series grew from November 10, 1925, to February 19, 1926, being transferred to the climatic chambers on January 26. The climatic data follow: Temperature, 13° to 15° C.; relative humidity, about 70 and 40 per cent., equivalent to approximately 3.8 mm. and 7.6 mm. respectively in saturation deficit; evaporation from the standard black atmometer, 82 cc. and 226 cc. respectively; thus, the evaporation in the drier atmosphere was 176 per cent. in excess of the other; maximal artificial illumination, 680 foot candles; total solar radiation, 5,140 calories in the house and 790 in the chamber; mean hourly maximum of solar radiation, 740 foot candles;¹⁰ mean hourly solar radiation (based on 8-hour day), in house 1,130 foot candles, (on 9-hour day) in chambers 510 foot candles.

TABLE XIV

YIELDS AND PROTEIN CONTENT OF SERIES 8. GROWTH PERIOD 102 DAYS. AVERAGE TOTAL ILLUMINATION OF 8-HOUR DAY, 1,140 FOOT CANDLES

RELATIVE HUMIDITY	NITROGEN SUPPLY	YIELD OF DRY GRAIN	PROTEIN CONTENT OF GRAIN	YIELD OF PROTEIN
Per cent.		Gm.	Per cent.	Cgm.
40	Liberal	2.06	18.2	38
70	Liberal	1.79	18.6	33
40	Scanty	1.87	18.6	35
70	Scanty	2.62	17.9	47

The results indicate a favorable effect upon seed yield of the higher plane of atmospheric humidity, with the nitrate supply limited. In this case, also, the protein yield was greatest. The differences in composition were relatively slight, however, and one may conclude that atmospheric humidity exercises much less influence than temperature upon the metabolism of wheat. This is in agreement with the observations of WASSILIEFF (19) upon *Lupinus albus*. The test should be repeated with greater illumination.

EFFICIENCY OF ILLUMINATION

For this test wheat and buckwheat were reared on soil in separate semi-circular pans of galvanized iron, constructed to fit the rotating tables of the chambers. In this way large numbers of plants were obtained per culture. The first crop grew from November 10 to 24, 1925, after emergence from the soil. The climatic data follow: Temperature, 16°-17° C.; relative

¹⁰ Adjusted for increased illumination before entering chambers.

humidity about 60 per cent.; artificial illumination, 340 and 680 foot candles; total solar radiation, 470 calories; mean hourly maximum of solar radiation, 760 foot candles; mean hourly solar radiation of 8-hour day, 550 foot candles; evaporation, 12 per cent. excess at the lower illumination. The analyses were performed on the undried tissues.

TABLE XV

RELATION OF ILLUMINATION TO DEVELOPMENT. GROWTH PERIOD, 15 DAYS. TEMPERATURE, 16-17° C. ARTIFICIAL ILLUMINATION OF 14-HOUR DAY, 340 AND 680 FOOT CANDLES

PLANT SPECIES	MEAN TOTAL ILLUMINATION, 14-HOUR DAY	DIMENSIONS OF STEM		LEAF PRODUCT	AVERAGE DRY WT. PER PLANT	NITROGEN CONTENT OF DRY MATTER
		LENGTH	DIAMETER			
	Foot candles	mm.	mm.	sq. mm.	mg.	Per cent.
Wheat	650	64	1.4	580	20	5.2
Wheat	990	61	1.5	760	23	5.4
Buckwheat ..	650	182	1.8	290	28	2.2
Buckwheat ..	990	191	2.6	420	19	3.9

The results appear in table XV. Both species of plants responded to the higher plane of illumination by increased leaf area, and the buckwheat attained a correspondingly greater diameter of stem. The yield of wheat increased somewhat without much change in nitrogen content. In the case of buckwheat, we attribute the low yield and high nitrogen content of the tissue produced with higher illumination to increased development of embryonic tissue from the seed reserves, with limited development of photosynthesis.

A second test was extended over a longer period, but in the lower solar light intensity of December 11 to January 4, 1926. Each culture supported 150 plants. The only differences in environmental conditions as compared with the preceding test were in solar radiation. These values were here as follows: Total, 570 calories; mean hourly maximum, 640 foot candles; hourly mean of 8-hour day, 400 foot candles. The appearance of the plants is shown in figure 3, and the yield and composition are given in table XVI.

The yields of dry matter indicate a more serious deficiency of illumination for buckwheat than for wheat. On the other hand, if one assumes the content of sucrose as storage material to be an index of light requirements, the wheat responded more than buckwheat to the increase of illumination. The contents of both total nitrogen and reducing sugars indicate that the lower plane of illumination was here inadequate.

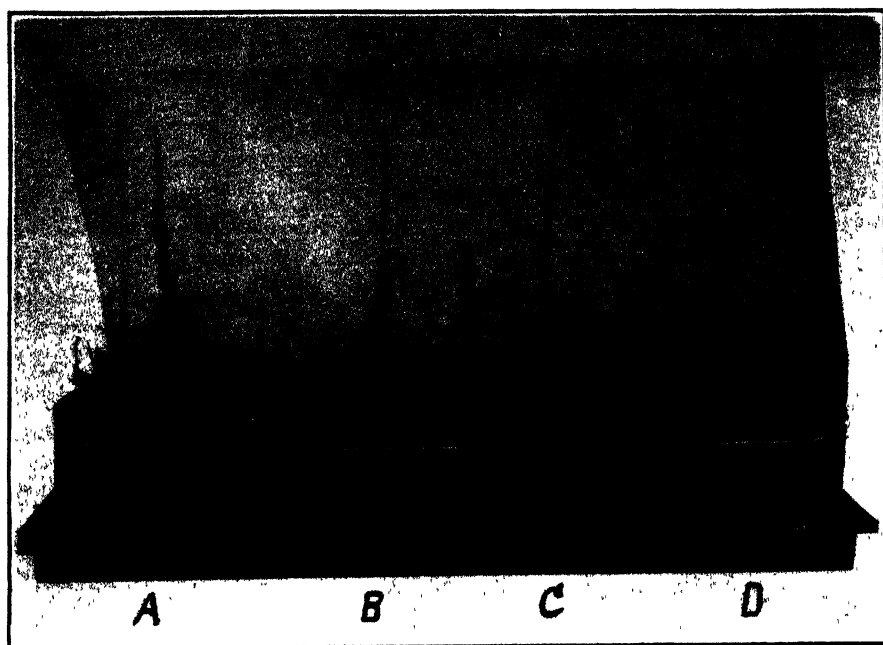


FIG. 3. Relation of illumination to development. Cultures of table XVI tied only for photographing. A. Wheat under higher illumination. B. Wheat under lower illumination. C. Buckwheat under higher illumination. D. Buckwheat under lower illumination.

TABLE XVI

RELATION OF ILLUMINATION TO DEVELOPMENT. GROWTH PERIOD, 25 DAYS. TEMPERATURE 16-17° C. ARTIFICIAL ILLUMINATION OF 14-HOUR DAY, 340 AND 680 FOOT CANDLES

PLANT SPECIES	MEAN TOTAL ILLUMINATION, 14-HOUR DAY	DRY MATTER	TOTAL NITROGEN OF DRY MATTER	REDUCING SUGAR OF DRY MATTER	SUCROSE OF DRY MATTER
	Foot candles	Gm.	Per cent.	Per cent.	Per cent.
Wheat	570	3.48	4.3	0.3	1.3
Wheat	910	4.20	4.1	0.9	2.1
Buckwheat	570	2.74	4.2	0.4	0.7
Buckwheat	910	4.59	4.0	0.9	0.8

Further tests are necessary to establish the minimum requirement of wheat under the conditions of our cultures. The above results indicate a possible deficit of illumination for wheat at about 1,100 foot candles over a 12-hour day. It will be necessary to consider the quality of the light also.

In these tests it was relatively deficient in the shorter wave lengths. We have preliminary evidence that these rays of sunlight or of the light from a carbon arc are important in the assimilation of nitrogen from nitrates.

TEMPERATURE EFFECTS IN YOUNG PLANTS

It might well be expected that one could detect in the composition of the young plant indications of such temperature effects as become pronounced at maturity. For investigating this point the tissues should be analyzed from the succulent condition, thus avoiding rearrangements likely to ensue in drying.

To test this matter wheat was grown in water cultures on the full and 0.2N solutions. Forty-five seedlings were reared in each 3.5 liter jar, employing the maximum of artificial radiation available. Growth covered the period March 5 to April 3, 1926, on the 0.2N solution, and 4 days longer on the full one. The effective climatic data were as follows: Temperature 13° to 15° C. and 18° to 20° C.; maximal artificial illumination, 850 foot candles; total solar radiation 2,040 calories and 2,250 calories for the respective sub-series; mean hourly maximum of solar radiation 1,270 and 1,340 foot candles, respectively; mean hourly solar radiation (basis of 10-hour day) 950 foot candles and 925 foot candles respectively; evaporation from standard atmometer 411 cc. at the lower temperature and 402 cc. at the higher, in the first sub-series, 447 and 451 cc. respectively in the longer test. Thus the conditions for evaporation were practically equivalent at the two temperature ranges.

A second test was run from April 19 to May 13 and 14, substituting 0.1N for the 0.2N solution. All factors excepting solar radiation were practically of the same values as in the preceding test. These data are as follows: Total solar radiation 2,400 and 2,450 calories at the respective end dates; mean hourly maximum solar radiation 1,630 foot candles to May 13; mean hourly solar radiation (basis of a 13-hour day) 1,040 foot candles; evaporation to May 13, 248 cc. at the lower temperature and 258 at the higher; thus the increase of evaporation was only 4 per cent. at the higher temperature.

Solar radiation during the measured absorption of nutrients were as follows: Test A, full solution, 324 calories; 0.2N, 333 calories. Test B, full solution, 498 calories; 0.1N, 428 calories. In test B the vacuolar sap and protoplasmic extract were separated by the method of CHIBNALL (2). In all cases the production of dry matter was greater at the higher temperature, as follows: April 3, low temperature 10.2 gms., high temperature 14.3 gms. April 7, low temperature 14.2 gms., high temperature 23.1 gms. May 13, low temperature 13.2 gms., high temperature 13.4 gms. May 14, low

TABLE XVII

COMPOSITION OF SEEDLINGS^a AND UTILIZATION OF NITRATE AT DIFFERENT TEMPERATURES. GROWTH PERIODS: TEST A, 30 DAYS; TEST B, 25 DAYS. AVERAGE TOTAL ILLUMINATION: TEST A, 1,600 FOOT CANDLES IN 13-HOUR DAY; TEST B, 1,890 FOOT CANDLES IN 13-HOUR DAY

TEST	PLANE OF NI-TRATE	TEMPER-ATURE ° C.	RED. SUGAR	SU-CROSE	PEN-TOSAN	INSOL-UBLE PROTEIN	SOLUBLE PROTEIN	NITRO-GEN BASES ^b	MONO AMINO ACIDS ^c	NITRATE	ABSORP-TION OF NITRATE
			Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
A	0.2N	13-15	6.0	2.2	0.8	10.0	5.5	0.9	5.6	2.3	60
A	0.2N	18-20	4.4	0.8	0.8	5.0	3.4	0.9	3.8	3.9	50
A	Full	13-15	2.9	12.1	0.8	10.6	3.1	0.8	4.2	3.9	44
A	Full	18-20	2.5	4.8	0.4	6.3	3.8	0.7	3.0	3.7	49
B (sap)	0.1N	13-15	1.9	4.1	0.6	0.5	0.2	1.5	14.3	74
B (sap)	0.1N	18-20	1.9	2.6	0.5	0.4	0.3	0.8	5.0	70
B (extract)	0.1N	13-15	0.4	0.4	0.9	15.1	1.0	0.5	1.5
B (extract)	0.1N	18-20	0.2	0.5	2.0	8.1	0.8	0.4	1.7
B (sap)	Full	13-15	2.1	4.8	0.5	0.4	0.6	2.5	4.6	-32 ^d
B (sap)	Full	18-20	1.7	3.9	0.6	0.4	0.6	1.9	4.3	-33
B (extract)	Full	13-15	0.6	2.4	0.8	10.2	1.4	0.3	1.3
B (extract)	Full	18-20	0.6	1.5	0.8	6.4	0.8	0.3	0.9

^a Percentages based on dry matter.

^b N of protein-free phosphotungstic acid precipitate $\times 5$.

^c N of filtrate from basic nitrogen $\times 5$.

^d Negative balance. 32-33 per cent. increase in the solution.

temperature 13.6 gms., high temperature 15.7 gms. The results of both series appear in table XVII.

These data show irregularities in trend but, on the whole, seem to justify the following generalizations: Sucrose and proteins were synthesized to a greater extent at the lower range of temperature. This was true to a lesser degree of reducing sugars and amino acids. A similar apparent dependence of nitrate assimilation upon the occurrence of sugars was noted in earlier work with the sugar beet (18). The absorption of nitrate at the lower planes of supply was favored by the lower temperature. At the higher plane of supply this relation seems to be reversed. The negative absorption of nitrate at the full plane of supply in test B is correlated with low solar illumination. On the day of sampling and the preceding day the mean hourly solar radiation in the chambers was only 500 and 420 foot candles respectively (1,350 and 1,270 foot candles total illumination). Soluble pentosans and basic forms of nitrogen formed relatively constant proportions of the tissue. As might be expected from the artificial mode of its determination, soluble protein shows no regular response to temperature. Indeed, the significance of attempts to distinguish between soluble and insoluble protein in such foliage extracts seems highly questionable. The protein content of the dry roots (insoluble and coagulable nitrogen $\times 6.25$) was determined for the full nutrient solutions in both series of cultures. The results were as follows:

April 7, low temperature 13.9 per cent., high temperature 13.3 per cent.
May 14, low temperature 12.1 per cent., high temperature 13.2 per cent.
These results are rather uniform.

Discussion

The preceding details give some metabolic responses of the wheat plant to rather completely circumscribed environmental conditions. Considering the limitations placed upon other factors, the degree of regulation of temperature was satisfactory. It appears that rather wide fluctuations of atmospheric humidity have little influence upon composition during maturation of the plant.

Illumination is in need of further investigation as regards both quality and intensity. Nevertheless, the tests here reported in which illumination was probably a limiting factor should possess some value inherent in a defined environment. The large proportion of sunlight employed entailed wide variations in total light intensity and distribution of wave lengths in the course of some tests, as well as in different culture series. In subsequent work it is purposed to employ light only from artificial sources. The results of these several series of cultures are summarized in table XVIII,

TABLE XVIII
SUMMARY OF CHIEF CONDITIONS AND RESULTS IN TEMPERATURE EFFECTS ON FULL NUTRIENTS

SERIES	PLANT PART	TEMPERATURE RANGE	AVERAGE PLANE OF TOTAL ILLUMINATION 12-HOUR DAY	DURATION	RELATIVE YIELD AND COMPOSITION AT LOWER TEMPERATURE AS COMPARED WITH HIGHER ONE					
					DRY MATTER YIELD	CARBOHYDRATES		PROTEINS		
						PERCENT-AGE	YIELD	PERCENT-AGE	YIELD	
		° C.	Foot candles	Days	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
1	Straw	10	390	41	155	170 ^a	265	100 ^a	150	
2	Straw	10	1010	41	90	68 ^a	61	135 ^r	117	
2	Roots	10	1010	41	144	140 ^b	200	87 ^r	123	
3	Straw	10	530	62	130	54 ^c	70	178 ^r	233	
3	Roots	10	530	62	119	90 ^c	107	67 ^r	78	
4	Straw	5	1150	80	324			95 ^{d,s}	309	
5	Seeds	6	1560	99	246			104 ^e	256	
5	Straw	6	1560	99	184			114 ^h	210	
6	Seeds	6	2510	82	152			80 ^e	122	
6	Straw	6	2510	82	112			77 ^h	86	
7	Seeds	8	830	85	104			83 ^e	88	
7	Straw	8	830	85	111			120 ^{d,s}	133	
A	Total extract	5	1730	30	61	205 ^d	126	136 ^{d,s}	84	
B	Sap	5	2050	25	104	123 ^d	107	100 ^r	87	
B	Extract	5	2050	25	104	143 ^d	124	161 ^{d,s}	140	

^a Hemicellulose.^b Total sugar and hemicellulose.^c Reducing sugar and hemicellulose.^d Total sugars.^e Crude protein.^f Insoluble protein.^g Soluble protein.^h Nitrogen compounds precipitable by phosphotungstic acid.

with illumination values converted to a uniform time basis. Without reference to the character of light, the following culture series were either certainly or possibly deficient in intensity of illumination, namely, 1, 3, and 2, 4, 7 respectively, with maxima of 530 and 1,150 foot candles over a 12-hour day. In most of these series the production of straw was decidedly favored by the lower temperature. The only crop in this group which produced seed (series 7) was reared outside the chambers. Its grain contained a considerably greater percentage of proteins at the higher temperature and a somewhat higher yield of protein. The other series (series 4) reared to maturity seems to have been disturbed in seed production by unfavorable temperatures for pollination, rather than by deficient illumination. At these lower planes of illumination the percentage of protein in the straw was generally favored by the lower temperature at the expense of available carbohydrates. Where carbohydrate determinations were omitted, it seems reasonable to assume that their percentages varied inversely as the larger differences of protein.

The remaining culture series (5, 6, A and B, with total light intensities of 1,560 to 2,510 foot candles over a 12-hour day) may be assumed to have received fairly adequate illumination, until proved otherwise. Plants harvested at one-third to one-fourth their full growth periods (series A and B) produced somewhat more tissue at the higher temperature. However, the increased percentages of sucrose and protein at the lower temperature gave evidence of increased storage capacity therefor. It seems probable that this function is determined by the plane of sugars, the latter then determining the plane of protein synthesis. This interpretation has support in the generally increased yield of protein, as well as of carbohydrates at the lower temperatures. The yield of both grain and straw from mature cultures (series 5 and 6) were greater at the lower temperature, the effect being marked with the grain. Increased proportions of carbohydrates in the tissues was also characteristic of the lower temperature.

The results here discussed apply to fully nourished plants. With a reduction in the supply of nitrate this nutrient generally became a limiting factor, especially at the higher temperature. In these several responses one generalization appears rather distinctly, namely: The accumulation of carbohydrates and proteins, as to quantity, is dependent upon growth at lower temperatures.

With increasing emphasis conditions have pointed toward the balance between synthesis and respiratory decomposition as the determinant of such compositional effects. The respiratory response is being measured. Meanwhile, LUNDEGARDH (13) has presented convincing evidence that this balance becomes critical with increase of temperature. It is well known that the

respiratory activity of plants increases with rise of temperature. The rate of increase is two-fold or greater at 15° to 25° C., while the increase of photosynthesis, as thus far determined, is appreciably smaller. Increase of temperature should thus result in decrease of the carbohydrate: protein ratio, as observed in the present work.¹¹ MCGINNIS and TAYLOR (15) take exception to this mode of interpretation, but the validity of their supposedly contradictory evidence is open to serious question. Working with detached seeds, they failed to take into account the determining effects of temperature upon the ratio of carbohydrate to protein in the reserves of leaf and stem, which supply the developing grain. MANGELS (14) concludes that the chief environmental factor influencing the percentage of protein in wheat is the temperature over several weeks preceding the filling stage. He found higher temperatures correlated with increased percentages of protein in the grain, although the yield of protein was less than at lower temperatures. Our experimental results agree with his statistical findings. The evidence seems to be strongly in favor of the conception that temperature becomes a dominant factor in the regulation of metabolism by controlling the equilibrium between certain anabolic and catabolic reactions. This conception does not preclude recognition of a possible rôle of aridity also in the production of high-protein wheat in cropping practice.

Summary

1. Improvements in apparatus for the control of environmental factors in plant growth are described, together with methods of estimating the total illumination effective from sun and electric lamps.
2. Details are given of the conduct and results with nine series of wheat cultures in water, sand and soil. Each series was subjected to two planes of temperature, but with illumination and atmospheric humidity uniform.
3. Evidence is presented that atmospheric humidity exercises little influence upon the protein content of wheat during the period of seed development.
4. With illumination probably deficient in intensity, lower temperatures favored the production of dry matter, as if conserving limited supplies of photosynthetic products. Under these conditions the lower temperature generally favored both the percentage and yield of protein in the tissues.
5. It is shown that the failure of seed formation in plants thus closely confined is chiefly due to unfavorable conditions for pollination.
6. With illumination probably adequate in intensity, young wheat plants contained greater percentages of sucrose and protein when grown at lower

¹¹ Since this was written BUSHNELL (Techn. Bull. 34, Minnesota Agricultural Experiment Station) has presented evidence that increased respiration at higher temperatures results in decreased tuber formation of the potato.

temperatures. Mature plants contained increased percentages of carbohydrates and gave markedly increased yields, especially of grain, at the lower temperatures.

7. The higher sugar content at lower temperatures appears to favor protein synthesis, but at higher temperatures deficiency of nitrate supply seems to operate as a limiting factor.

8. Although the amount of protein synthesized is less at the higher temperatures its percentage in the tissue is increased, apparently because of greater respiratory loss of carbohydrates than at the lower temperatures.

9. In explanation of these metabolic effects the conception is advanced that there is a critical balance between the temperature responses of photosynthesis and respiration.

10. The need of measurement of respiration and photosynthesis in continuance of this work is recognized. Furthermore, the light factor should be made reproducible as to both quality and intensity.

DEPARTMENT OF AGRICULTURAL CHEMISTRY,
UNIVERSITY OF WISCONSIN

LITERATURE CITED

1. BLACKMAN, F. F. Optima and limiting factors. *Ann. Bot.* **19**: 281–295. 1905.
2. CHIBNALL, ALBERT CHARLES. A new method for the separate extraction of vacuole and protoplasmic material from leaf cells. *Jour. Biol. Chem.* **55**: 333–342. 1923.
3. Contributions from the Boyce Thompson Institute for Plant Research. Vol. 1, no. 1. 1925.
4. GERICKE, W. F. Influence of temperature on the relations between nutrient salt proportions and the early growth of wheat. *Amer. Jour. Bot.* **8**: 59–62. 1921.
5. ————. Further notes on the growing of wheat in one-salt solutions. *Soil Sci.* **15**: 69–73. 1923.
6. HENDRICKS, ESTEN, and HARVEY, R. B. Growth of plants in artificial light. II. Intensities of continuous light required for blooming. *Bot. Gaz.* **77**: 330–334. 1924 (with erratum Vol. 78, p. v. 1924).
7. HOAGLAND, D. R., and DAVIS, A. R. Further experiments on the absorption of ions by plants, including observations on the effect of light. *Jour. Gen. Physiol.* **6**: 47–62. 1923.
8. HOTTES, CHAS. F. A constant humidity case. *Phytopath.* **11**: 51. 1921.

9. JONES, L. R. Soil temperature as a factor in phytopathology. *Plant World* **20**: 229-237. 1917.
10. KIMBALL, HERBERT H. Solar radiation intensities at Mount Weather, Va. *Monthly Weather Rev.* **42**: 138-141. 1914.
11. ———. Photometric measurements of daylight illumination on a horizontal surface at Mount Weather, Va. *Monthly Weather Rev.* **42**: 650-653. 1914.
12. LIVINGSTON, B. E., ed. A plan for cooperative research on the salt requirements of representative agricultural plants. Second ed. Baltimore, 1919.
13. LUNDEGARDH, HENRIK. Der Kreislauf der Kohlensäure in der Natur. Pp. 106-110. Jena, 1914.
14. MANGELS, E. E. Effect of climate and other factors on the protein content of North Dakota wheat. *Cereal Chem.* **2**: 288-297. 1925.
15. MCGINNIS, F. W., and TAYLOR, G. S. The effect of respiration upon the protein percentage of wheat, oats, and barley. *Jour. Agr. Res.* **24**: 1041-1048. 1923.
16. TOTTINGHAM, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. *Physiol. Res.* **1**: 133-245. 1924.
17. ———. Temperature effects in plant metabolism. *Jour. Agr. Res.* **25**: 13-30. 1923.
18. ———, LEPKOVSKY, S., SCHULZ, E. R., and LINK, K. P. Climatic effects in the metabolism of the sugar beet. *Jour. Agr. Res.* **33**: 59-76. 1926. (P. 70.)
19. WASSILIEFF, N. Eiweiss Bildung in reifenden Samen. *Ber. d. bot. Ges.* **26a**: 454-467. 1908.

SULPHUR METABOLISM OF YEAST¹

H. SUGATA AND FRED C. KOCH

Introduction

Two general conclusions are warranted as to the sulphur metabolism and nutrition in animals. The first is that although most of the sulphur taken into the system is in the reduced form, that is, mainly as cystine or possibly also as cysteine, nevertheless the main end product of normal sulphur metabolism is the completely oxidized sulphate form. This is well established by numerous investigations. The other important conclusion is that cystine is a very important amino acid in growth and maintenance.

Recently the importance of sulphur in nutrition of plants has also been so well shown that the use of sulphates in fertilizers is emphasized. ARMSTRONG (1) also studied the effect of various sulphur compounds in the development of *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea*. He concluded that the best form of food sulphur is sulphate, and that even here the end product of sulphur metabolism is the sulphate form.

Recent studies (7) in this laboratory indicate that cystine also may be of some importance in yeast growth and that studies on yeast, when properly controlled, may throw more light on certain phases of metabolism. The object of this investigation was to determine more definitely the forms of sulphur available for yeast growth, and to investigate further the changes cystine undergoes in yeast growth and metabolism.

In all the studies here reported the concentration of the yeast growth stimulant was carefully controlled just as in the work by MILLER (5) and SWOBODA (7). In the first part of the work various forms and amounts of sulphur, as found in biological material, were added to an artificial medium containing a known, but very small amount of sulphur, and the rate of yeast growth determined by the weighing method. In the second part of the study the yeast was separated and analyzed for sulphur, and the filtrate analyzed for total sulphur and for "inorganic sulphate."

Experimental

1. MEDIA AND SULPHUR COMPOUNDS.—The following stock media were used from time to time with or without other additions as indicated under the observations made. The materials used in these media and the various

¹ From the Hull Laboratories of Physiological Chemistry and Pharmacology, The University of Chicago.

sulphur compounds added were for the most part prepared or purified in these laboratories.

The saccharose first employed was the commercial rock candy, but we soon found this to contain sufficient amounts of sulphur to influence the results. It was then purified by solution in water and precipitation by redistilled absolute alcohol. By this procedure the sulphur content was reduced from the equivalent of 0.7 mg. to 0.1 mg. barium sulphate per gram of saccharose.

The asparagine was of the C.P. grade but was again recrystallized from water. The inorganic salts used were the "analyzed chemically pure" grade. The cystine was obtained from horn in the usual way and then reprecipitated five times from an aqueous solution of the hydrochloride by ammonium hydroxide. Cysteinic acid was prepared from cystine by FRIEDMANN'S method (2). It was recrystallized from hot water and washed with alcohol. Taurocholic acid was prepared from ox bile by HAMMARSTEN'S method (3), and taurine therefrom by the HAMMARSTEN (4) and TAUBER methods (8). The purity of these substances as measured by the sulphur content is indicated in table I.

TABLE I
SULPHUR CONTENT OF MATERIALS USED

SUBSTANCE	BaSO ₄ OBTAINED PER GRAM	PER CENT. OF SULPHUR	THEORETICAL PER CENT. OF SULPHUR
	mg.		
Purified saccharose	0.1		
Asparagine	0.2	...	
Vitamine solution 5 per cent. ...	0.085	...	
KH ₂ PO ₄	1.4		
Cystine	26.59	26.69
Cysteine	26.59	26.47
Taurocholic acid	5.33*	6.22
Taurine	25.0	25.63
Cysteinic acid	18.88	18.87
Dried yeast	0.35	

* The low value is probably due to an admixture of moisture and metallic impurities. An ash estimation confirmed the latter interpretation.

The vitamine solution was prepared by drying Fleischmann's best grade of stock yeast in a current of air at room temperature. This material was next thoroughly ground in a mill and extracted six times with hot absolute

ether. The ether extract was discharged and the yeast powder next extracted six times with boiling 75 per cent. alcohol. After cooling, the combined alcoholic extracts were filtered and then concentrated to a paste under diminished pressure. The material was next redissolved in hot 90 per cent. alcohol and again cooled in the refrigerator and filtered. Thus 500 cc. of stock solution were obtained from five pounds of fresh yeast. The vitamine solution as added to the media in most cases was a 5 per cent. solution of this alcoholic stock solution. The media used are shown in table II.

TABLE II
COMPOSITION OF MEDIA EMPLOYED

SUBSTANCE	MEDIUM NUMBER						
	1	2	3	4	5	6	7
	grams	grams	grams	grams	grams	grams	grams
Saccharose	20.0	20.0	20.0	20.0	20.0	100.0	100.0
Asparagine .. .	1.5	1.5	1.5	..	1.5	7.5	7.5
NH ₄ H ₂ PO ₄	3.0
KH ₂ PO ₄ .. .	2.0	2.0	2.0	2.0	2.0	10.0	10.0
MgSO ₄	1.20
MgCl ₂	0.25	0.25	0.25	..	1.01	..
CaCl ₂	0.25	0.25	0.25	0.25	1.25	1.25
Diluted to ..	1000	1000	1000	1000	1000	500	500

2. GROWTH METHOD.—The methods of WILLIAMS (9), MILLER (5), and SWOBODA (7) were used with slight modifications. A 125 cc. volume of the medium in a 500 cc. Erlenmeyer flask was sterilized in an autoclave at ten pounds for ten minutes and after cooling 1 cc. of the usual yeast suspension was added. After growth at 30° C. for 20 hours a few drops of formaldehyde were added so as to stop the growth of yeast. The yeast cells were at once filtered off into the weighed Gooch crucible, dried in the oven for 30 minutes at 103° C. and weighed after cooling.

3. ANALYTICAL METHOD FOR SULPHUR.—The method of alternate oxidation by hydrogen peroxide and by nitric acid as devised by STOCKHOLM and KOCH (6) was employed. It gave excellent checks and theoretical values for the well purified sulphur compounds and is very satisfactory for small amounts of sulphur.

Experimental observations

A. THE IMPORTANCE OF CALCIUM, MAGNESIUM AND SULPHATE IN YEAST GROWTH.—To 100 cc. of medium no. 1 varying amounts of M/10 CaCl₂, M/10 MgCl₂, M/10 MgSO₄, M/10 H₂SO₄ and vitamine solution were added

in various combinations and then diluted to 125 cc. Yeast was then grown in these special media, filtered off, dried and weighed. The results given in table III are with the unpurified saccharose and 5 cc. of the 5 per cent. vitamine solution per 100 cc. medium no. 1. In table IV we have a similar procedure, only here we have one-fifth of the amount of vitamine solution. In table V we have similar observations on medium no. 1 with the purified saccharose. Similar results were obtained repeatedly in connection with many other growth tests.

TABLE III

GROWTH OF YEAST IN UNPURIFIED SACCHAROSE, WITH 5 CC. VITAMINE IN MEDIUM NO. 1
WITH VARYING SALTS

NUMBER	MgCl ₂ M/10	CaCl ₂ M/10	MgSO ₄ M/10	(NH ₄) ₂ SO ₄ M/10	H ₂ SO ₄ N/30	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	cc.	mg.
1	2	2		.		17.7
2	.	2	2	...		49.1
3	2	..	1	44.2
4	.		.	2	1	1.6
5	2	2	.		1	48.5
6	..	2	2	2		51.0
7	2		2		1	47.4
8	2	2	.	2	.	42.1
9	2	2	2	.		52.2
10	.	2	2	.	1	48.4

TABLE IV

GROWTH OF YEAST IN UNPURIFIED SACCHAROSE, WITH 1 CC. VITAMINE IN MEDIUM NO. 1
WITH VARYING SALTS

NUMBER	CaCl ₂ 1%	MgCl ₂ 1.5%	MgSO ₄ 1.5%	(NH ₄) ₂ SO ₄ 2%	H ₂ SO ₄ N/30	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	cc.	mg.
1	2	2		.		9.6
2	2	..	2	.	..	12.1
3	2	.	1	1	.	12.8
4	2		2	1		14.4
5	2	.	2		1	12.3
6	2	..	4	.	..	11.8
7	2	4	...	1.2
8	2	...	8	11.8
9	2	8	...	1.4
10	2	..	4	4	..	14.6

TABLE V

GROWTH OF YEAST IN PURIFIED SACCHAROSE, WITH VITAMINE IN MEDIUM NO. 1 PLUS SALTS

NUMBER	CaCl ₂ M/10	MgCl ₂ M/10	MgSO ₄ M/10	H ₂ SO ₄ N/30	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	mg.
1	2	7.0
2	2	2	7.6
3	2	...	1	...	8.6
4	2	..	2	...	9.9
5	2	2	2	...	11.2
6	2	...	4	...	11.4
7	2	..	8	..	9.7
8	2	2	...	1	9.7
9	2	2	...	2	10.2

B. THE SUBSTITUTION OF REDUCED SULPHUR FOR SULPHATE SULPHUR IN THE MEDIUM.—In the first part of these studies the cystine was dissolved in a KH_2PO_4 solution and this was then added to a modified medium no. 3 together with a standard KH_2PO_4 solution so that the total amount of KH_2PO_4 always remained 2.0 grams per liter. Many series of growth tests were made and in all cases the results were essentially as indicated in table VI. However, if asparagine is not present in the medium the results in

TABLE VI

GROWTH OF YEAST IN MEDIUM NO. 3, PLUS VITAMINE AND CYSTINE AS INDICATED WITH AND WITHOUT ASPARAGINE

NUMBER	VITAMINE SOL. 5 PER CENT.	CYSTINE	WEIGHT OF DRIED YFAST WITH ASPARAGINE	WEIGHT OF YEAST WITHOUT ASPARAGINE IN THE MEDIUM
	cc.	mg.	mg.	mg.
1		...	2.9	0.5
2	1	.	8.9	2.5
3	1	1	10.0	...
4	1	2	10.5	2.2
5	1	3	12.4	...
6	1	4	14.3	2.5
7	.	1	3.0
8	..	2	3.6	1.3
9	..	3	4.2
10	..	4	5.1	1.3

the last column of table VI are obtained. By adding various mixtures of the salts and cystine to medium no. 6, together with vitamine in each case,

the values in table VII were obtained. Practically the same observations are given in table VIII, where medium no. 6 also was used as the stock medium with 1 cc. vitamine solution in each test.

TABLE VII

GROWTH OF YEAST IN MEDIUM NO. 6, PLUS VITAMINE WITH SALTS AND CYSTINE ADDED, AS INDICATED

NUMBER	MgCl ₂ M/10	MgSO ₄ M/10	NH ₄ Cl M/10	CYSTINE	AVERAGE WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	mg.	mg.
1	2	9.2
2	2	1	10.7
3	2	3	11.4
4	..	2	14.4
5	...	2	...	1	14.9
6	.	2	...	3	14.5
7	2	..	2	...	11.2
8	2	.	2	1	11.7
9	2	.	2	3	12.0
10	2	..	20	1	10.4
11	2	..	20	3	11.2

Cysteine, used in amounts ranging from two to twenty milligrams, gave results very similar to cystine.

TABLE VIII

GROWTH OF YEAST IN MEDIUM NO. 6, PLUS 1 CC. VITAMINE, AND WITH SALTS AND CYSTINE ADDED

NUMBER	MgCl ₂ M/10	MgSO ₄ M/10	NH ₄ Cl M/10	CYSTINE	WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	cc.	mg.
1	2	8.6
2	2	.	..	1	10.2
3	2	3	13.9
4	2	10(?)	10.4
5	.	2	14.3
6	.	2	...	1	16.5
7	.	2	..	3	13.3
8	...	2	...	10(?)	12.7
9	2	..	20	...	8.2
10	2	...	20	1	10.9
11	2	..	20	3	12.8
12	2	...	20	10(?)	12.4

Hydrogen sulphide seems to be able to supply sulphur for yeast growth to a limited extent. The results, using medium no. 6 as the stock medium, are given in table IX. In each case 1 cc. of the vitamine solution was added.

TABLE IX

GROWTH OF YEAST IN MEDIUM NO. 6, PLUS 1 CC. VITAMINE, WITH SALTS, AND H_2S ADDED AS INDICATED

NUMBER	MgSO ₄ M/10	MgCl ₂ M/10	H ₂ S 1 cc. = 0.00216 mg.	WEIGHT OF DRIED YEAST
	cc.	cc.	cc.	mg.
1	2	13.1
2	2	..	1	10.8
3	2	..	2	10.9
4	2	..	4	10.2
5		2	..	8.4
6		2	1	10.7
7		2	2	11.0
8		2	4	9.9
9		2	6	10.2
10		2	10	7.3
11		2	20	6.9

C. THE VALUE OF SULPHONATE SULPHUR.—In tables X and XI are given the results obtained with taurocholic acid, cysteinic acid and taurine. In table X are given the results, using medium no. 3 and no. 4 as stock media. In table XI the stock medium was no. 5.

TABLE X

GROWTH OF YEAST WITH TAUROCHOLIC ACID AS SULPHUR SOURCE

NUMBER	5 PER CENT. VITAMINE SOLUTION	TAUROCHOLIC ACID ADDED	MEDIUM NO. 4 WEIGHT OF DRIED YEAST	MEDIUM NO. 3 WEIGHT OF DRIED YEAST
	cc.	mg.	mg.	mg.
1	1	0	8.5	12.3
2	1	1	8.6	11.5
3	1	4	6.3	10.2
4	1	10	5.0	7.1
5	0	0	2.6	3.0
6	0	1	2.5	1.8
7	0	4	1.6	1.5
8	0	10	1.1	1.3

TABLE XI

GROWTH OF YEAST IN MEDIUM NO. 5, CYSTEINIC ACID AND TAURINE TESTS

NUMBER	5 PER CENT. VITAMINE SOLUTION	MgCl ₂ M/10	MgSO ₄ M/10	CYSTEINIC ACID	TAURINE	AVERAGE WEIGHT OF YEAST
	cc.	cc.	cc.	mg.	mg.	mg.
1	1	2	9.0
2	1	2	...	2	...	10.6
3	1	2	...	6	..	10.0
4	1	2	...	10	...	9.9
5	1	2	...	20	...	9.7
6	1	2	2	9.3
7	1	2	6	9.5
8	1	2	.	..	10	9.7
9	1	2	.	..	20	10.2
10	1	...	2	.	..	14.9
11	1	...	2	2	..	13.4
12	1	.	2	6	...	11.0
13	1	...	2	10	...	10.4
14	1	.	2	20	.	9.9
15	1	...	2	..	2	14.6
16	1	..	2	...	6	14.6
17	1	.	2	..	10	13.9
18	1	..	2	.	20	14.3

D. UTILIZATION OF INORGANIC SULPHATE SULPHUR.—Yeast was grown in 500 cc. of medium no. 7 with the addition of 5 cc. vitamine solution. After the usual growth period the yeast was separated by centrifuging and washed twice by centrifuging and decanting. After drying and weighing, the total sulphur was estimated by the method referred to previously. The filtrate was carefully recovered and the "total sulphate" estimated by the usual method. The original medium contained 0.0244 grams sulphur as sulphate; the filtrate from the yeast contained 0.0225 gram or a difference of 0.0019

TABLE XII

UTILIZATION OF CYSTINE SULPHUR BY YEAST, MEDIUM NO. 6

SUBSTANCE	TOTAL SULPHATE	TOTAL SULPHUR	CYSTINE SULPHUR	SUM
	gm. S	gm. S	gm. S	gm. S
Original medium	0.00137	0.0127	0.01407
Yeast filtered off (0.6752 gram)	0.00462	0.00462
Filtrate from yeast	0.00541	0.00322	0.00863

gram sulphur. This amount apparently was removed from the medium during growth. The yeast filtered off contained 0.0016 gram sulphur.

E. UTILIZATION OF CYSTINE SULPHUR.—In this case 0.05 gram cystine was added to 500 cc. of medium no. 6 and the sulphate, cystine and total sulphur determined in the original medium and in the filtrate after growth. The cystine was determined by the phosphotungstic acid precipitation method as employed in the VAN SLYKE procedure for protein analysis. The results are given in table XII.

Discussion of results

A comparison of tables III and IV first of all shows the stimulating action of increased amounts of vitamine under otherwise constant conditions. Both of the tables very clearly show the value of both magnesium and sulphate in yeast growth; and it is particularly noteworthy to observe that in the presence of a liberal supply of the growth-stimulating vitamine, the effect of the same addition of magnesium and sulphate is eight to ten times that observed with a low vitamine content. The results also show that the optimum concentration of magnesium is very soon attained even where growth is extensive as shown in table III. An addition of magnesium beyond that amount neither improves nor injures the medium, no matter whether the vitamine concentration is low or high. The same general conclusions hold for sulphate. Comparison of no. 4 and no. 3 in table III very strikingly shows how much more important magnesium is in this medium than the ammonium. Calcium does not appear to be nearly so important as magnesium.

The observations on the effect of sulphate clearly show that growing yeast is able to utilize sulphate and presumably convert it into protein (probably cystine) sulphur. The quantitative studies on the loss of sulphate from a sulphate medium confirm the same general conclusions as to the utilization of sulphate in yeast growth.

The studies with cystine again confirm the slight stimulating effect thereof when in the lower concentrations, but that one can soon observe an inhibiting effect when the concentration is increased beyond say five to ten milligrams per 125 cc. of medium. This inhibiting effect is observed much more readily when sulphate also is present in the medium. In a sulphate-free medium an otherwise minimal inhibiting concentration of cystine is still stimulating. So also the stimulating effect of cystine is much more marked in a medium containing asparagine than in one free therefrom. In no case, however, could cystine sulphur be satisfactorily substituted for sulphate sulphur. An amount of cystine, equivalent in sulphur content to a good concentration of sulphate as magnesium sulphate or ammonium

sulphate, is toxic in any case. Thus ten milligrams of cystine contain 3.6 milligrams of sulphur whereas 6.4 milligrams of sulphur as sulphate is a very favorable concentration. The observations on cysteine lead to the same general conclusions.

Hydrogen sulphide can also be drawn upon for yeast growth in the absence of sulphate, but one soon reaches a toxic concentration which is far below the sulphur content of a desirable sulphate concentration. Whether the sulphide itself is exceedingly toxic and only that part of it which has become oxidized in the medium is utilized by the yeast, or whether a trace of the sulphide is the real intermediate form between sulphate and yeast tissue sulphur is not determined. Here again an extremely low concentration of hydrogen sulphide (0.00216 milligram H_2S per 125 cc.) is toxic to yeast growth when sulphate is added to the medium, but when the sulphate has not been added it requires ten times the concentration of hydrogen sulphide before a toxic effect is observed. However, even then the stimulating effect is very slight indeed. The action of hydrogen sulphide is very similar to that of cystine, but it is very much more toxic. Possibly cystine itself is harmless, but hydrogen sulphide liberated from it in traces may be the real reason for the apparent toxicity of cystine.

Apparently the yeast is able to protect itself against cystine to some extent by oxidizing the sulphur in cystine to the sulphate form. This is shown in table XII. This observation is very interesting because the yeast metabolism of excess cystine appears to be very similar to that in man where the main excretory form is sulphate. The results also indicate that of the cystine sulphur which was changed, approximately equal amounts were found in the yeast as yeast protein and in the filtrate as sulphate respectively.

The results with taurocholic acid, cysteinic acid and taurine indicate that taurocholic acid is toxic in any case no matter whether other more available forms of sulphur are present or not. Cysteinic acid aids only slightly in an otherwise sulphur-free medium; and in the other media, containing more available forms of sulphur, it is toxic. Cysteinic acid thus stands between cystine and taurocholic acid. Taurine is without appreciable action in the concentrations employed. These results suggest that the cholic acid part in taurocholic acid introduces the toxic effect, but also that the sulphonate form of sulphur is the least available for yeast growth.

Summary and conclusions

1. Magnesium and sulphate appear equally important in yeast growth.
2. Of the forms of sulphur studied, the inorganic sulphate form is the most available.

3. Sulphate sulphur is a true nutrient in that it is actually converted into yeast protein and probably at least in part into cystine.

4. Cystine, cysteine, and hydrogen sulphide stimulate yeast growth in a sulphate-free medium up to certain, but low concentrations. Above those concentrations they retard growth. Cystine also stimulates slightly in a sulphate-containing medium, but hydrogen sulphide retards growth in such a medium even in extremely low concentrations.

5. When yeast grows in a sulphate-free medium containing cystine as the source of sulphur it converts part of the cystine into new yeast protoplasm and about an equal amount into sulphate left in the medium.

6. Taurocholic acid in very low concentrations retards yeast growth. Cysteinic acid acts intermediate between taurocholic acid and cystine.

7. Taurine is without action on yeast growth in the concentrations here employed.

HULL LABORATORIES OF PHYSIOLOGICAL CHEMISTRY AND PHARMACOLOGY,
UNIVERSITY OF CHICAGO

LITERATURE CITED

1. ARMSTRONG, G. M. Sulphur nutrition: The use of thiosulphate as influenced by hydrogen-ion concentration. *Ann. Missouri Bot. Gard.* **8**: 237-281. 1921.
2. FRIEDMANN, E. Über die Konstitution des Cystins. *Beitr. z. chem. Physiol. u. Path.* **3**: 1-46. 1903. Method p. 29.
3. HAMMARSTEN, O. Über die Darstellung kristallisierter Taurocholsäure. *Zeitschr. physiol. Chem.* **43**: 127-144. 1904.
4. ———. Untersuchungen über die Gallen einiger Polarthiere. *Zeitschr. physiol. Chem.* **32**: 435-466. 1901. Method p. 456.
5. MILLER, ELIZABETH W. The effect of certain stimulating substances on the invertase activity of yeast. *Jour. Biol. Chem.* **48**: 329-346. 1921.
6. STOCKHOLM, MABEL, and KOCH, FRED C. A quantitative method for the determination of total sulphur in biological material. *Jour. Amer. Chem. Soc.* **45**: 1953-1959. 1923.
7. SWOBODA, F. K. Nitrogen nutrition of yeast. *Jour. Biol. Chem.* **52**: 91-109. 1922.
8. TAUBER, S. Über einige Derivate des Taurins und die Synthese der Taurocholsäure. *Beitr. z. chem. Physiol. u. Path.* **4**: 323-330. 1904.
9. WILLIAMS, R. J. A quantitative method for the determination of vitamine. *Jour. Biol. Chem.* **42**: 259-265. 1920.

STUDIES ON THE OXIDATION OF CERTAIN FATTY ACIDS

J. B. RHINE

Introduction

In both plant and animal bodies, under certain conditions, sugars are changed to fats; and in the plant, if not in the animal, these fats are converted back to sugars for use when a certain other set of conditions is provided. These are well known facts of physiology. It is pretty well established that the point of departure is the fatty acid; that is, the fatty acid is formed before the neutral fat, and the neutral fat is hydrolized to fatty acid and glycerol before being changed to sugar. Thus we may consider the changes as one between fatty acid and sugar for our present purposes. This reversible transformation is one that is very common and widespread in the plant kingdom, not to mention the animal world. Great quantities of food are put through this process, especially in the ripening and germination of fatty seeds. In these cases the carbon is conducted as a soluble carbohydrate and then stored in the lighter, more compact, and insoluble form of fat. Before being transportable again and ready for use in other parts of the plant it must be made soluble again; the common transport material of the fatty seedling is sugar.

But these very common food transformations are not understood. We know that at one time we have sugar and at another, fat, or vice versa; but we do not understand how this remarkable change can be so generally brought about especially at the normal air temperatures. This general biological interchangeability of fats and sugars has no chemical parallel, nor have chemists been able to point out the manner in which such a transformation can be made, although it is a question of much simpler compounds, structurally, than some that have been successfully dealt with in the laboratory. Of course, we know that the formation of the fatty acid requires a reduction of the carbon atom, and the change back to sugar is an oxidation process; and we know further that in so changing, it has attached an atom of oxygen per carbon atom. Anything further on the intermediate steps is purely hypothetical and not supported by evidence.

The fact that enzymes have been isolated which are found to aid in the reactions of many of the known plant processes, naturally suggests that there may be one or more enzymes responsible for the changes here under consideration. It must be remembered, however, that there is probably much to protoplasmic activity that is not to be detached, so to speak, not

performed by separate agents like enzymes. No doubt some of the more fundamental processes are inherent in the very nature and structure and activity of the protoplasm itself. The sugar-fat-sugar processes may be of this order. At any rate, this must be borne in mind in the interpretation of negative results in searching for enzymes for these processes.

The most logical attack on the general problem seemed to be an attempt to discover if an enzyme could be isolated which would bring about a part or all of these changes *in vitro*. It seemed best, too, to work with the fatty acid-to-sugar half of the process for the following reasons: That the oxidation phase seemed likely to occur more easily *in vitro*; that the products would probably be more easily tested for; that starting with long chain fatty acids involved less danger of contamination with organisms than starting with sugars; etc. Obviously, even this single phase of finding an enzyme which changes fatty acids to sugars represents an extensive undertaking and probably could never be settled in the negative, so many are the possibilities to be exhausted. The enzyme, if any, can only be sought for with such general methods as are available, adding such special modifications as may occur, and continuing the search until it no longer seems profitable, or until it is found.

Experimental

In 1923 while at Hull Botanical Laboratory of the University of Chicago an attempt was made to find an enzyme responsible for this reaction. Brief mention of this attempt is made by way of introduction to what is to follow. Mixed fatty acids from linseed oil were used chiefly, although not entirely. The extractions for the enzymes were made for the most part upon germinating fatty seeds, and in general the methods of extraction were those used to prepare lipase, although other methods were tried, even to using the filtered and dialyzed expressed juice of the seedlings. Small amounts of fatty acids were put in small flasks, with the water solution of the extract slightly acidified with HCl (using 1 part of fatty acid to about 10 parts of extract solution), shaken up frequently, and kept in a 40° C. oven. In some cases thymol, in others chloroform, and in others nothing was added to prevent the activity of organisms. No signs of such activity were ever present however. A check on the changes going on in the extract itself was made in each case, and a check on the fatty acids also was made each time, using acidified distilled water in place of the extract. They were allowed to stand for various periods of time, up to three weeks. The preliminary test for results used was reduction of Fehling's solution by the water layer in the flask.

No reduction of Fehling's solution was obtained, however, which was not also obtained from the check flask that did not contain the extract.

Only a slight amount of reducing substance was present at all, but the same degree of reduction was produced in the flask containing only the fatty acids and the acidified distilled water. The amount of substance was too small to use in further testing for its nature. But, although the experiments failed in their main objective, they made evident the fact that these fatty acids were undergoing a decomposition of some kind, yielding a water soluble, reducing substance. It could not, of course, be called a sugar on such evidence, but the facts were sufficient to warrant further investigation of the nature of this substance, and the process by which it was produced. The enzyme work was merely laid aside then, not settled.

In 1924, at the Boyce Thompson Institute for Plant Research a study was undertaken of the decomposition of fatty acids which was apparent from the enzyme experiments mentioned. The object was still to throw some light on the processes of the plant by which fatty acids are changed to sugar; the immediate aim was to study the products yielded in this mild oxidation, considering that they might lead to some understanding of the break down of the fatty acid in the organism. The general method was to furnish higher temperatures, increased acidity, and abundance of oxygen to the mixture of fatty acids and water, the water being used to remove the soluble products as they were formed. The method is analogous to the general method for performing, *in vitro*, hydrolyses that normally occur in the organism, substituting for the enzyme, heat and acidity.

To provide these conditions, 100 ml. of the fatty acids and 200 ml. of distilled water (in some cases made up to N/50 sulphuric acid, later in most cases not acidified) were put in an 800-ml. Kjeldahl flask fitted with a reflux condenser. Tube connections leading from a compressed air tank ran to the bottom of the flask so that the air bubbled up continually and kept the two liquids well mixed. The flask was kept at a roughly constant temperature, varying at most 3° C., by means of a sand-bath on an electric hotplate. All runs were made between 75° and 95° C. The current of air, in addition to emulsifying the oil and water, furnished a continually fresh supply of oxygen. In some cases a rapid stirrer was used, with only a slow current of air. This, however, furnished no advantage over the other method of mixing (rapid bubbling of air) and was discontinued. After a few attempts the sulphuric acid also was left out, since it seemed to make no great difference; the water solution soon became acid without it. The strong current of air had the disadvantage of carrying off some of the more volatile products, even through the long reflux condenser. But it was found that most of the volatile products could be caught by connecting with the upper end of the reflux condenser and leading the out-going air through a flask of water, rendered slightly alkaline.

The fatty acids used were prepared from purest raw oils obtainable. They were carefully saponified with alcoholic potash and the fatty acids freed by addition of dilute sulfuric acid to the hot diluted soap. The free acids were repeatedly washed until the washings showed no sign of the mineral acid. The fatty acids were tested for acrolein and for unsaponified fat (Geitel test). These impurities were never found in any of the fatty acids used. The palmitic and stearic acids were obtained on the market, the best available. They were not resaponified because by the time they were used it was clear that the neutral fat was not objectionable in small quantities in this investigation. This is made evident later.

After the treatment of the fatty acids for the desired period, the water layer was separated from the fatty layer by filtering. For general testing, filtering through ordinary filter paper was sufficient; for certain cases, however, it was necessary to use charcoal. Other filters such as asbestos, aluminum crucibles, etc., were tried. It was difficult to completely remove all the finely divided and suspended oil droplets from the solution without the use of charcoal.

The first general test on the products of this treatment of the fatty acids was made with Fehling's solution. As a preliminary test equal parts of the mixed Fehling's solution and of the solution to be tested were put into a test tube and heated in a boiling water bath for 15 minutes. For the quantitative determination of the amount of reduction, the Schaffer and Hartman iodometric modification of the Munson and Walker method was used. When much of the product was present, the solution reduced the copper very distinctly in the cold. In all cases some reduction was obtained from all fatty acids tried, after sufficient treatment. Considering the reaction only from the standpoint of the amount of reducing substance produced, there are at least three statements that may be made: (1) That the reaction increased with temperature and time, within limits, as would be expected; (2) that the fatty acids from linseed oil yielded more reducing substance than the neutral oil; (3) that the reducing substance increases with the degree of unsaturation of the fatty acids used. The preliminary fashion in which this phase of the problem was attacked allows only these three points to be made, although several others are fairly evident; it will bear much further work.

The quantitative data available to show the increase of reducing substance with temperature and time (up to a certain limit) appears in table I. In addition to this many estimates made in the test tube in a standard fashion, and carefully estimated, support these figures substantially.

Inasmuch as these determinations were incidental to the main trend of the research they were not carried out in duplicate; they are therefore not

TABLE I

A. EFFECT OF TEMPERATURE RISE ON YIELD FROM MIXED LINSEED FATTY ACIDS

TEMPERATURE °C.	DURATION OF TREATMENT	MGMS. OF COPPER REDUCED PER 100 ML.
Room = 23-25	2 weeks, shaken twice daily. No other mixing.	Distinct trace
40	2 weeks, shaken twice daily. No other mixing.	11.5
65	30 hours, mixed by air	42.0
80	30 hours, mixed by air	48.0
95	30 hours, mixed by air	47.6

B. EFFECT OF TIME OF TREATMENT, TEMPERATURE CONSTANT

95	20 hours, mixed by air	45.0
95	30 hours, mixed by air	47.6
95	72 hours, mixed by air	47.0
95	90 hours, mixed by air	44.0

published as absolutely exact, but the estimates made in the test tube bear out the general trend beyond question. In addition to these data, rough estimates made at intervals of 2 hours with temperature constant at 95° C., showed an increase up to 20-22 hours, after which the amount of reducing substance remained fairly constant. It became apparent that, after the point was reached at which about 45 to 48 mgms. of Cu. would be reduced per 100 ml. of solution, the concentration of reducing substance was not increased. It has not yet been determined what the limiting factor is, although it is likely to be volatility, since part of the reducing substance distills over at 100° C., and the current of air together with the high temperature carries over much volatile material.

TABLE II

COMPARISON OF YIELD OF REDUCING SUBSTANCES BY NEUTRAL LINSEED OIL WITH LINSEED FATTY ACIDS

SUBSTANCE	TEMP. ° C.	TIME	MLS. OF N NaOH USED TO NEUTRALIZE 25 ML. SOLUTION	MGMS. CU RE- DUCED PER 100 ML.
1. Neutral oil	95	20 hours	0.3	6.6
2. Fatty acids	95	20 hours	1.1	45.2
3. Fatty acids . .	95	90 hours	1.67	44.0

The second statement, that the free fatty acids of linseed oil yielded more reducing substances than the same amount of neutral oil, is based upon the figures in table II.

It may also be seen from this table that in cases 1 and 2 the greater yield of reducing substances is accompanied by a greater production of acidity of the solution. This is a general phenomenon in these experiments, until after the maximum concentration of the reducing substance is reached; thereafter the acidity increases slowly while the reducing power does not. Case 3 in table II illustrates this. After 90 hours at the same temperature it had no more reducing action, but had more acidity.

Thirdly, it was stated that the greater the degree of unsaturation of the fatty acids, the greater the yield of reducing substances under the same conditions. This is illustrated for several fatty acids in table III.

TABLE III
RELATION BETWEEN UNSATURATION AND YIELD

No.	FATTY ACIDS	IODINE VAL.	TEMP. ° C.	TIME IN HOURS	MGMS. CU PER 100 ML. SOLUTION
1	Linseed fatty acids.....	170.8	80	30	48.0
2	Cottonseed fatty acids.....	109.4	80	30	26.0
3	Stearic acid (E & A).....	10.4	85	90	10.6

When time is considered the results here show striking differences between fatty acids having different degrees of saturation. Oleic acid belongs between stearic and cottonseed fatty acids, as shown by test tube estimates of reducing power. A purer grade of stearic acid was obtained and used in other experiments, but no quantitative measurement of the amount of reducing compounds yielded was made on this. It had an iodine value of 1.22, yet it still furnished reducing substances under this treatment, though in smaller quantities, as measured by the estimation of the precipitate in the test tube.

The main object of these studies, however, was to find out how close to the carbohydrate condition the products of this mild oxidation approached. One of the chief tests for simple carbohydrates was satisfied in the reduction of copper; the substances also reduced ammoniacal silver, without heating. Further, they gave a positive aldehyde test with the fuchsin-SO₂ test, gave a negative ketone and positive aldehyde reaction with thiobarbituric acid, and positive aldehyde with iodoform and nitroprusside tests. It seemed necessary, then, to conclude that we were dealing with water-soluble aldehydes. It seemed probable since they were water soluble that

they were not long chain fatty aldehydes, *i.e.*, longer than nonylic aldehyde; and since they lacked the strong characteristic odors, they could not be short chain fatty aldehydes. The assumption was made then that the reducing compounds had more oxygen than that of the carbonyl group. This was in the direction of the carbohydrate structure.

The Molisch test for carbohydrates (α -naphthol) gave neither clear-cut positive nor negative results. Different observers pronounced it a "light" test, a faint reddish-violet color, in this case nearly obscured by a heavy brown smudge.

Also, in all cases with all fatty acids tried, from purified stearic with an iodine value of 1.22 to the most unsaturated, the solution from the treatment was in some degree fermentable with bread yeast. Although this did not mean necessarily that the compounds were carbohydrates, the restricted limits into which this reaction threw them made it seem worth while to test out this phase thoroughly. It was found first, that when the treatment was made without a reflux condenser, there were left reducing substances, but no fermentable compounds. Distillation tests showed that this fermentable fraction was carried over at 94° to 99° C. Furthermore, it was not distillable from neutral or alkaline solution, but passed over when the solution was re-acidified and redistilled. It was evidently an organic acid of short length. There was some evidence to show that this compound loses its fermentability when evaporated to dryness on the steam bath; of course this was done in slightly alkaline solution to prevent loss of the substance by evaporation. After fermentation, iodoform tests were made on the distillate from the fermentate, rendered alkaline for distillation. The object was to see if ethyl alcohol was produced by the fermentation, carbon dioxide having been the only measure used till then. Iodoform precipitate was obtained from this distillate, but it formed in the cold, indicating acetone, isopropyl alcohol, or an allied substance, but not ethyl alcohol. Nitroprusside tests for acetone, however, were negative. An iodoform precipitate was obtained in the cold from the distillate from an alkaline solution of the original unknown solution before fermentation; it was also obtained from the distillate made from this alkaline residue after acidifying and redistilling. Negative nitroprusside tests showed that acetone was not present either. It is evident, therefore, that there is more than one substance yielding iodoform present in the original solution, one an acid, one other at least not acid. To give iodoform, the acid would have to have either a hydroxyl group or a double-bonded oxygen in addition to its carboxyl group. Also, it is probable from the knowledge available on fermentation that an organic acid, to be fermentable by bread yeast, must have additional oxygen in its structure, and probably must be an alpha-hydroxy- or alpha-ketonic-acid.

In this respect the iodoform and fermentation results agree. It would appear then that we have here an hydroxy or ketonic acid of such length that it distills from its water solution near the boiling point of water.

It was found that the fermentable compound (or compounds) was separable from the main part of the aldehydes which furnished the reducing action, by distillation. A large portion of the aldehydes was carried over from slightly alkaline solution which retained the fermentable part. The reducing power was much diminished by prolonged heating, especially in alkaline solution, and the residue readily took on a charred appearance with a crisp physical character. We know, therefore, that the aldehyde portion is not the fermentable substance; that it is in part carried over from its boiling solution, and partly destroyed in the distillation; that it probably contains more oxygen than the fatty aldehydes, since it has not the characteristic strong odors possessed by acetaldehyde, propionic, butyric, heptylic, nonylic, and other fatty aldehydes.

With this mixture, it was obviously difficult to work with the phenylhydrazine reactions. Considerable effort was spent with phenylhydrazine and modified phenylhydrazines, and while some results were obtained, they were not consistent. It was quite clear that none of the osazones of the carbohydrates were obtainable. This line of attack was then left until methods of purification and concentration without destruction should be perfected for this special mixture.

In the endeavor to separate out components of the mixture, their solubility in alcohol and ether was tested. The original solution was evaporated to dryness slowly, and the residue extracted first with ether, second with absolute alcohol, and third with distilled water, all at room temperature. This distilled water extract is designated as S_1 . After the three extractions were made considerable residue (R_1) remained. Also, when the ether and alcohol extracts were freed from the ether and alcohol and 20 ml. distilled water added to take up the soluble portions, (S_2 and S_3), considerable remained as residue (R_2 and R_3). To each of the three residues 2 ml. of $N NaOH$ were added; the $NaOH$ dissolved the ether and alcohol residues (R_2 and R_3), and a part of the water extraction residue (R_1). These (S_4 , S_5 , and S_6) were made up to volume equal to the extractions and all were compared as to reducing action, fermentability, and Molisch test.

The ether extraction removed most of the fermentable substances, although small amounts were left in the alcohol, and in the residue. But none of these substances were taken up by the water, until after the hydroxide was added, showing again that the fermentable portion is an acid. It is likely, too, that the evaporation to dryness causes a change in these acids,

a polymerization of some sort; this is suggested to explain why they are water soluble at the outset but are not after the evaporation. Also, the fact that a resinous type of residue results from evaporation to dryness, suggests polymerization, although, of course, we do not know from that which compounds are affected here.

The ether extract contained about one fourth of the total reducing substances, most of this fourth going into the water added after the ether was removed (S_2). The alcohol extract, following the ether extraction, contained about one tenth of the total reducing power, most of it being water soluble (S_3) again after the alcohol was evaporated off. The remainder of the reducing substances, the greatest part of them, was found in the water extraction (S_1) taken on the original products following the ether and alcohol extractions. A trace was found in the residue left (R_1) after all the extractions, and it was liberated by the addition of the hydroxide. These results were obtained from duplicate attempts made along this line, and were the same in each case. As mentioned above, these extractions were made at room temperature. By shaking ether with the original solution, from one fourth to two thirds (approximately) of the reducing power could be taken up, depending on the way the original solution was prepared. Three washings with fresh ether were made in each case. This again indicated that there were two kinds of reducing substance present. The best Molisch test (for carbohydrates and like compounds) given was obtained from the high reducing fraction, the water extract (S_1). It was positive, but not a strong test. No other of the separations gave the test at all.

The ether extract gave an odor of burnt sugar, combined with a fruity odor. "Maple sugar," "fudge," "burnt candy," etc., were suggested by different individuals who smelled it. It proved to be attractive to flies also. Molds grew on solutions from the ether extract, also on the alcohol extract which contained the fermentable compounds; and care had to be taken with all the original solutions made from any of the fatty acids used, since solutions from all became contaminated with molds upon standing at room temperature.

An attempt was made to get a measure of the length of chain of one of the acids present in the mixture which was carried over from its water solution at the boiling point, by means of its neutralization value. I was not sure of the purity of the compound, and was not sure of its oxygen content. Assuming it to be of the acetic series it would have to be an 8-carbon chain, or more probably a mixture of 7 and 9-carbon chains. However, it is very likely that there were other volatile impurities which would invalidate these figures and that there is more oxygen in the molecule than in the acetic series.

Several compounds were tested for, which had been reported in the literature as coming from the decomposition of fatty acids under certain conditions, different from those used in these experiments, but in general conducing to oxidation of the molecule. Thus, acrolein, acetone, epihydrin aldehyde, pyruvic acid, and lactic acid, were tested for and all were found to be absent from the solution obtained from the oxydation by this method. Certain of the strong smelling fatty aldehydes, such as butyric, heptylic, nonylic, etc., were known to be absent by the absence of the rancid odor they are known to impart to certain old fats.

From these studies it can be seen that much is yet to be done before we can speak with certainty on the manner of breakdown of these fatty acids under these conditions; the conditions are simple—oxygen, water, temperature (mild), and the decomposition of the acids should more likely take place at the weakest points than if the conditions were more drastic. More study of the products must be made, also, but certain statements can be made on the basis of these experiments that will at least serve as suggestions to other workers in this particular phase of fat chemistry. First, we can say that all fatty acids yielded a fermentable acid, not of the acetic series, and not pyruvic nor lactic acid. It is ether- and water-soluble and distills from its water solution, going over with the first fraction. It seems to polymerize readily on drying. Second, these same fatty acids yield with the same treatment water soluble aldehydes, not fermentable with yeast. They reduce Fehling's solution, and give various specific aldehyde tests. Some, at least, can be distilled from their water solution. They tend to char and are easily destroyed. The insolubility of some of these reducing substances in ether indicates the presence of more oxygen than the one atom in the carbonyl group. The fact that they were in great part insoluble in absolute alcohol and yet were carried over in part during distillation of their solution requires further work for explanation. Possibly only the ether soluble and alcohol soluble portion was distillable. It should be mentioned here again that the best Molisch test for carbohydrates obtained at all was given by this water-soluble-only aldehyde substance. This is the point nearest to the carbohydrate group arrived at in these investigations. Third, it is evident that more than one aldehyde was produced, some being aromatic, others (one other at least) not.

Changes in fatty acids during treatment

The fatty acids were found to undergo some very decided changes during the course of these treatments, and with a view to getting more light on the nature of the decomposition reactions, these fatty acids were studied before and after the treatment by the standard methods of examining fats,

viz.: specific gravity, saponification value, neutralization value, lactone value, iodine value, acetyl value, etc. Results from such examinations are shown in table IV.

TABLE IV

DATA ON CHANGES OCCURRING IN FATTY ACIDS DURING TREATMENT

No.	TEST	LINSEED FATTY ACIDS		STEARIC ACID (E & A)	
		BEFORE	AFTER	BEFORE	AFTER
1	Specific gravity	0.9079	1.0112	0.8902	0.9114
2	Saponification value	194.4	236.3	215.87	223.60
3	Neutralization value	194.4	144.4	204.3	193.1
4	Lactone or anhydride value . .	1.6	91.9	11.54	30.5
5	Iodine value	170.8	73.5	10.4	2.93
6	Acetyl value	68.03	114.8		

In addition to these changes, the viscosity of the liquid fatty acids increased during treatment. The increased specific gravity resulted in the oil layer being at the bottom of the flask in which the treatment occurred instead of at the top, after sufficient oxidation had taken place. This was found only in the linseed fatty acids. This increase of specific gravity is undoubtedly due to the absorption of oxygen; the decrease of the iodine value bears this out, and the increase of the acetyl value shows that part of this extra oxygen taken on is retained as hydroxyl groups. The increase in the saponification value indicates an increase in the number of carboxyl groups per unit weight, caused by the formation of shorter chain fatty acids. The fact that the neutralization value did not follow the saponification value, and the fact that the lactone value increases so markedly during treatment gives evidence of the formation of lactones or of polymerization products, in either case yielding water and forming an anhydride. This is dependent upon the presence of hydroxylated fatty acids and is further evidence of the formation of these. In fact it is necessary to consider both the acetyl value and the lactone value in getting an estimate of the degree of hydroxylation of the fat, since the formation of the lactones would lower the acetyl value in the same degree in which they were formed.

Although some of the changes produced in the fatty acids are similar to those occurring in rancidity of fats, blowing of oils, and other conditions under which oils have been oxidized, the products are quite different in this case from those obtained in the other methods of treatment. All products reported in the literature as resulting from other methods of oxidation have been tested for, the outstanding ones especially, such as acetone, fatty ketones, acrolein, butyric aldehyde, butyric acid, epihydrin aldehyde,

heptylic and nonylic aldehydes, etc., and have not been found present in detectable quantities in the solutions resulting from this method. It is thought that this method has the virtue of allowing a mild oxidation to occur in water solution (except for the first stages, which of course occur in the fat itself), the water entering into the reaction and yielding a different group of products from those just mentioned. Most experiments along this line have either had no water present or have been too drastic to secure a breakdown along lines that approach the biochemical change we are studying. No doubt pure oxygen, used instead of air for the blowing through the mixture of fatty acids and water, would hasten the oxidation; and perhaps many things may be added to accelerate the reaction, particularly the ordinary mineral catalysts. But these have not been tried in this maiden attempt because it was considered best to solve first the problems brought up by the simple treatment as it was, before introducing new complications of uncertain value. Hastening the oxidation may well change the reaction and the products entirely, and these modifications should, therefore, form another problem for later consideration.

Summary

1. Fatty acids when treated in the manner described in this paper undergo a mild oxidation, in which they absorb oxygen, become hydroxylated, and break up into shorter-chain acids, some soluble in water and some not, and into aldehydes, soluble in water; they probably yield other fragments, too. This breakdown differs in its products from all other reported methods of oxidizing fatty acids, and probably approaches more nearly to the conditions obtaining in the plant, though still far from them.

2. The products remaining in the water solution after this treatment, at any rate approach more nearly the sugars in structure than the products reported in the literature from other methods of oxidation. Water-soluble, ether-insoluble, non-volatile aldehydes which have more oxygen than that of the aldehyde or carbonyl group have been found; the actual structure of these is not known but it is considered an approach toward the carbohydrate condition, and toward the solution of the problem of explaining the common biological interchangeability of the fats and sugars.

3. In addition to these aldehydes there are some that are both water soluble and ether soluble, and are distilled from water solution.

4. Short chain acids are produced which are water soluble, ether and alcohol soluble, can be distilled from their water solution, and are fermentable by bread yeast, yielding carbon dioxide and fermenting in about the same time as glucose with the same yeast. Molds grew on these solutions, but only on the fermentable ones.

Change of the writer to a different field of endeavor not involving laboratory connections is responsible for the appearance of this report of an undertaking that is obviously unfinished. It is made, however, largely for its suggestive value and it is hoped that in this respect at least it will justify its being added to the scientific literature. Chief among those to whom I am indebted for helpful discussion in this work are: Professor F. C. KOCH and Professor C. A. SHULL, of the University of Chicago, Dr. S. H. ECKERSON, Boyce Thompson Institute, and Dr. LOUISA E. RHINE. The generous efforts of Dr. P. D. STRAUSBAUGH in providing time and materials for continuing work at West Virginia University are gratefully acknowledged. The proper citation and discussion of the enormous literature on the various branches of science touched upon in this paper would more than double its size. Reasonable brevity is the excuse for its omission.

BOTANY DEPARTMENT,
WEST VIRGINIA UNIVERSITY,
MORGANTOWN, W. VA.

THE DISTRIBUTION OF THE ROOTS OF SUGAR CANE IN THE SOIL IN THE HAWAIIAN ISLANDS

H. ATHERTON LEE

(WITH SIX FIGURES)

Introduction

Up to the present time several excellent excavation studies of the roots of sugar cane and other crop plants have been made, from which observations of considerable value have been secured. It is generally appreciated, however, that in the biological sciences quantitative determinations are more valuable than statements of observation and for that reason the methods outlined in this paper were developed. These methods with slight modifications are feasible for use with a number of crop plants, so that they may be of interest to other investigators in the plant sciences. An examination of the stimulating studies by WEAVER (3), and his associates, in which the literature of root studies has been extensively reviewed, indicates that the methods employed in the present studies have not been previously in use, to any general extent at least.

In growing sugar cane or almost any crop, a large proportion of the cost of producing the crop is chargeable to the operations of tillage in preparation for root growth. It is obvious that this investment in tillage can be more intelligently made if it is known where the roots of the crop will extend or where they may be made to extend. Moreover a knowledge of the distribution of the roots enables one to prevent their injury by cutting or mutilation during the operations of cultivation. Determinations of soil moisture may be confined to the levels of the soil where the roots exist, if it can be determined where the large proportion of the roots become distributed. Irrigation applications can be more economically applied, and water conserved if it is known where the roots exist. The greatest efficiency in the use of fertilizers depends on placing them where they will be quickly available to the greatest number of roots. This is a concise statement of some of the more ready applications of knowledge of root distribution to economical and efficient crop production. WEAVER (2) has discussed in greater detail many applications of this knowledge to the growth of plants.

The root-study-box methods

Our first method consisted in growing sugar cane in wooden boxes, thirty by thirty inches square and thirty-six inches deep; in these boxes wire poul-

try netting with a $1\frac{1}{2}$ inch mesh was placed horizontally at different depths and the sides of the boxes were detachable. The boxes were then filled with soil and the cane planted. When the cane reached an age at which it was desirable to make the determinations, the sides were taken from the boxes, the soil was washed away with a stream of water, and the roots were exposed but held in their correct position of growth by the horizontally placed poultry netting. This is more readily understood from the illustration shown in fig. 1 than from the written description. These root-study boxes are somewhat similar to the earthenware cylinders used and described by VENKATRAMAN (1).

With the roots exposed and available for examination in this way, the first attempt to secure a quantitative expression of their distribution was by linear measurement. However, as in several previous attempts at root studies, this attempt to measure the length of the roots was not a success due to the large number of secondary and tertiary roots which made such measurements a tedious and very nearly impossible task. For this reason we adopted the procedure of weighing the roots rather than measuring their length. To do this the roots were cut off at different levels in depth, collected together from a given level, washed, oven-dried, and weighed. The first results obtained by these methods are shown in table I which follows:

TABLE I

DISTRIBUTION OF THE ROOTS OF FIVE-MONTHS-OLD CANE, VARIETY D 1135, IN TWO TYPES OF SOIL. THREE PLANTS AVERAGED FOR EACH SOIL TYPE

DEPTH IN THE SOIL	ROOTS OF ALL PLANTS AVERAGED		ROOTS OF PLANTS IN LOOSE SANDY LOAM		ROOTS OF PLANTS IN COMPACT LOAMY CLAY	
	Grams	Per cent.	Grams	Per cent.	Grams	Per cent.
Topmost 8 inches ...	75.5	63.9	50.4	64.3	100.6	64.6
8 to 16 inches ...	22.6	19.1	15.7	20.1	29.4	18.9
16 to 22 inches ...	8.6	7.3	6.3	8.0	10.9	7.0
22 to 30 inches ...	11.3	9.6	6.0	7.6	14.7	9.5
Totals ...	118.0	99.9	78.4	100.0	155.6	100.0

Although these results were an advance over the previous lack of quantitative data there was considerable doubt as to their validity for field conditions due to the abnormal aeration of the soil in boxes and the more frequent irrigation and presence of wire netting in the boxes. Moreover the roots of cane could only be studied up to a certain age because of the size of the boxes. For this reason a method for use under field conditions was developed.

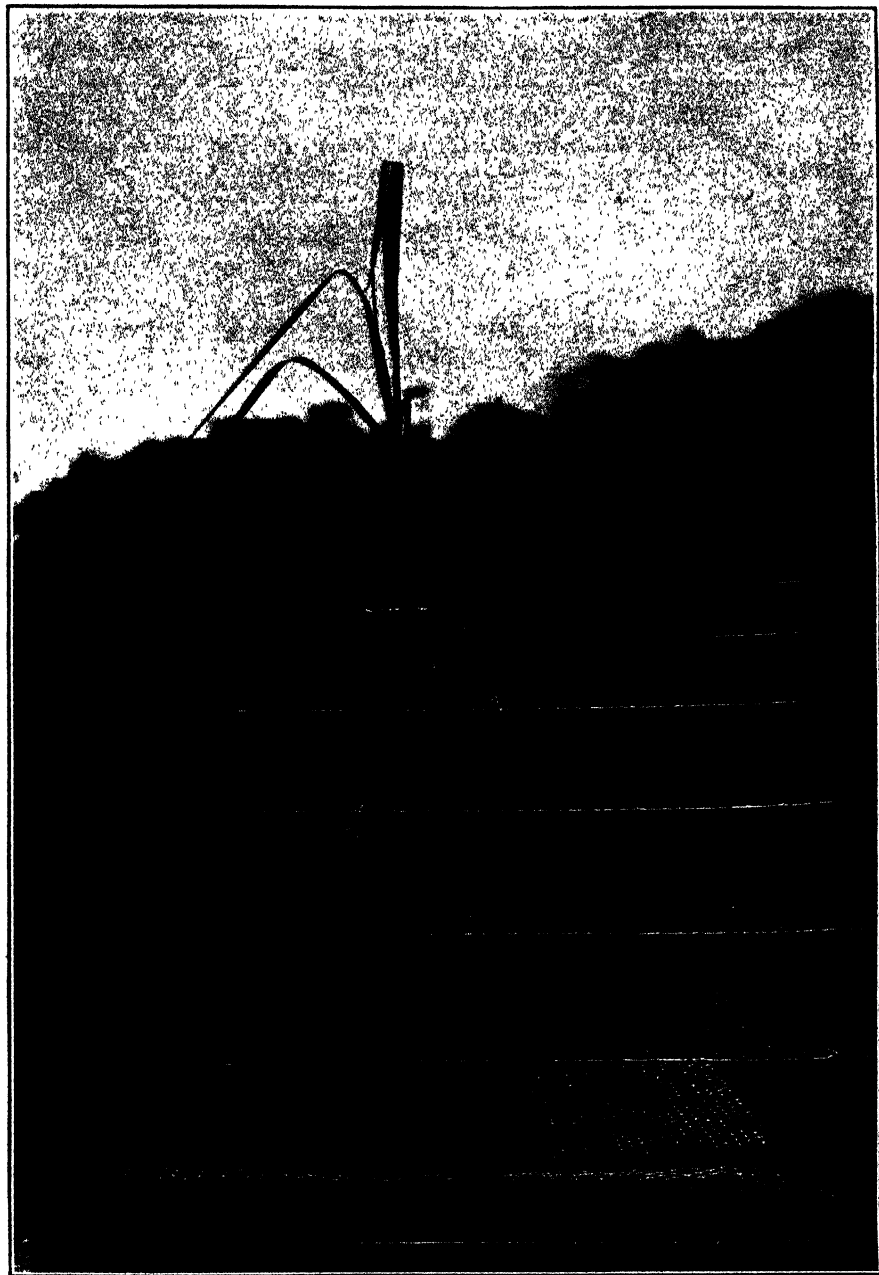


FIG. 1. Root-study box with detachable sides removed and the soil washed away leaving the roots of the plant held in position by the horizontally placed wire netting. Sugar cane, variety H109, one month after planting.

Methods for quantitative root studies under field conditions

In this method as in the method in root-study boxes the principle of weighing the roots rather than linear measurement was resorted to, since dry root weights are fully as good an index of absorbing surfaces as linear measurements when we are dealing with fibrous roots, and the root weights are readily obtained; whereas linear measurements are so difficult to make as to be impractical.

An area in the field was selected in which representative stools of cane could be found for study. The outermost stools of cane in a row were always avoided since they may so easily be abnormal due to the greater amounts of air, light or irrigation water at the edge of the field. In all cases the determinations were made on five stools of cane and the results averaged per stool.

The five stools of cane to be studied having been selected, the cane was cut down to the juncture of the cane stalks with the soil; this point of juncture was arbitrarily called the surface of the soil. Then from the middle of the cane stools a distance was measured out on either side to the centers of the spaces between the rows. Since the cane studied up to the present has been in all cases in five-foot rows, the distance measured from the centers of the stools was 2.5 feet. Stakes were then driven in at these center points between the rows, forming a rectangle, including the five stools to be studied. In order to form a base mark for measurements of the depths of subsequent excavations, light boards were nailed to these corner stakes and brought to a uniform level with the aid of a spirit level.

At the edge of the rectangle to be excavated a wire screen of quarter-inch mesh, such as is used for screening sand for mortar or cement, was placed in a sloping position. Excavation was then started within the rectangle containing the five stools to be studied and was carried to a level which we arbitrarily fixed in these studies at eight inches below the surface of the soil. As the soil was excavated it was thrown on the wire screen standing at one side, and as the soil was sifted through the screen the roots were separated from the soil and were collected in paper bags. When an excavation reached a level of eight inches, and the roots down to that level had been collected and bagged, a further excavation was made to a depth of sixteen inches and the roots between eight and sixteen inches in depth collected and bagged separately. Further excavations were made to levels of 24, 32, and 40 inches below the surface of the soil or as far down as appreciable quantities of roots were found.

When the excavations reached a depth where only negligible quantities of roots were found, each separate collection of roots was washed, oven-dried and weighed. These weighings gave a quantitative expression of the



FIG. 2. The excavation, from different levels of which the roots have been collected. At either end of the pit is shown the base mark from which measurements are taken to establish the different levels in depth in the excavation. At the right is shown the wire mesh screen through which the excavated soil from the different levels is sifted and the roots separated out.

amounts of roots at the different levels in depth in the soil. An illustration of an excavation in progress is shown in fig. 2.

For different purposes, of course, these levels in the soil need not be always eight inches in depth but may be six or ten inches or whatever is found to give results of the greatest value in application. Roots of the plants being studied, which extend beyond the rectangle excavated, are compensated by the roots of the cane in the parallel rows on either side, so that there is only a slight error if any from such a source. It is appreciated, in heavy soils especially, that there is a loss of very small roots through the wire screen of quarter-inch mesh. However, the proportion of error from this source must be very nearly the same at all levels in the soil, therefore this source of error is not regarded as affecting the results seriously.

No cane stubble was included in the weighing; where the roots were dug up attached to the cane stubble, the roots were separated away with a knife and the stubble discarded.

Some results with this method

The first determination with this method was made on the variety H 109, plant cane, 10.5 months old. This cane had had 28 irrigations and had received 275 pounds of nitrogen and 175 pounds of phosphoric acid per

acre, but no potash. The quantities of roots obtained at the different levels in depth of the soil are shown in table II.

TABLE II

FIRST DETERMINATION OF THE QUANTITIES AND DISTRIBUTION OF THE ROOTS OF CANE IN THE SOIL. THE ROOTS STUDIED WERE OF 10.5 MONTHS-OLD PLANT CANE OF THE VARIETY H109

DEPTH IN THE SOIL	AVERAGE WEIGHT OF ROOTS PER STOOL IN GRAMS	PERCENTAGE OF TOTAL ROOTS
Topmost 8 inches.. . . .	135.23	70.14
8 to 16 inches	43.92	22.78
16 to 24 inches	11.34	5.88
24 to 30 inches.. . . .	2.29	1.18
Totals	192.78	99.98

These results are shown graphically in the photograph of the root masses from the different levels, reproduced in fig. 3.

One can see that these results check fairly closely with the results from the root-study boxes recorded in table I, that is, although the total root weights per plant varied considerably in the two studies, the percentages of the total roots in the different levels in depth were not very divergent. In both studies unexpectedly high percentages of the roots were found in the upper eight inches of soil and more than ninety per cent. were found in the upper twenty-four inches of soil. It seemed necessary to substantiate these results and build up a considerable mass of evidence to prove or disprove the presence of such a large proportion of the roots in the uppermost layers of soil. In the following tables are presented the results of a number of root excavations and weighings; the results are in every case the averages of five stools of cane.

In table III are shown the root weights of stools of cane of the Yellow Tip variety from two different fields; in the first field the cane averaged 35 tons per acre as compared to 75 tons of cane per acre in the second field. Both fields were unirrigated, 26 months old at the time of the excavations, and had received identical fertilizer treatment.

In the foregoing table one may observe that although the total roots per plant varied in the two fields as much as forty per cent., the percentages of the roots in the different levels of the soil were very nearly identical for the two fields. As in the preceding excavations also a very large proportion of the roots was in the upper eight inches of soil, in both cases being seventy per cent. or more.

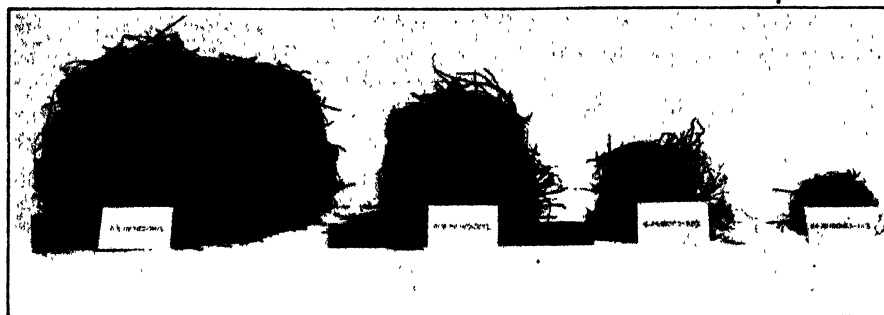


FIG. 3. Comparative masses of roots of the variety H109 sugar cane from different levels in depth in the soil. The root mass at the left is from the topmost 8 inches, and root masses of graduating size to the right are from the lower levels in depth, in sequence. The root mass in the topmost eight-inch layer of soil is a lighter brown color than the masses from greater depths, apparently because of the presence of a much larger proportion of secondary feeding roots near the soil surface which are lighter in color than the primary roots.

TABLE III

COMPARISON OF ROOTS OF CANE OF THE YELLOW TIP VARIETY IN A FIELD WHICH YIELDED 35 TONS OF CANE PER ACRE, WITH THE ROOTS OF CANE OF THE SAME VARIETY IN A FIELD WHICH YIELDED 75 TONS OF CANE PER ACRE

DEPTH IN THE SOIL	ROOTS PER STOOL OF CANE YIELDING 35 TONS PER ACRE		ROOTS PER STOOL OF CANE YIELDING 75 TONS PER ACRE	
	Grams	Per cent.	Grams	Per cent.
Topmost 8 inches	72.5	70	107.3	73
8 to 16 inches	20.3	19	26.1	18
16 to 24 inches	8.7	8	11.6	7
24 to 32 inches	2.9	3	2.9	2
Totals	104.4	100	147.9	100

To extend the evidence as to the distribution of the roots of cane in the soil, two further excavations were made on another plantation, as shown in table IV.

A comparison of the root distribution of the two different varieties is not intended in table IV; the results are grouped together merely for economy of space. These results are in close agreement with those of the previous excavations, showing high proportions of the roots in the uppermost layers of the soil. The results with the seven-months-old Yellow Tip cane illustrate a point which will be developed in a subsequent publication, that young plants have higher proportions of roots in the surface layers of soil than plants of greater age; in other words, the center of gravity of the root masses goes lower with increasing age.

TABLE IV

QUANTITIES AND DISTRIBUTION OF THE ROOTS OF 7-MONTHS-OLD YELLOW TIP CANE AND 11-MONTHS-OLD BADILA CANE

DEPTH IN THE SOIL	SEVEN-MONTHS-OLD CANE OF YELLOW TIP VARIETY		ELEVEN-MONTHS-OLD CANE OF BADILA VARIETY	
	Grams	Per cent.	Grams	Per cent.
Topmost 8 inches	26.1	75	31.9	68.75
8 to 16 inches	5.8	17	8.7	18.75
16 to 32 inches	2.9	8	5.8	12.50
Totals	34.8	100	46.4	100.00

Two additional excavations at different plantations, although they afford no direct comparison of roots in different soil types, still are in agreement with a thesis which has been developed in these studies: That, in general, larger proportions of the roots of sugar cane extend to the lower levels in a soil of a sandy loose texture than in more compact loam or clay soils. The results of these two excavations are shown in table V.

TABLE V

QUANTITIES AND DISTRIBUTION OF ROOTS OF 16-MONTHS-OLD CANE IN HEAVY ADOBE SOIL, AND 11.5-MONTHS-OLD CANE IN LOOSE SANDY LOAM, VARIETY H109, BOTH UNDER IRRIGATION

DEPTH IN THE SOIL	16-MONTHS-OLD H109 IN HEAVY ADOBE SOIL		11.5-MONTHS-OLD H109 IN LOOSE SANDY LOAM	
	Grams	Per cent.	Grams	Per cent.
Hilled-up soil between the rows			25.82	3.90
0 to 8 inches	44.02	75.5	61.87	54.96
8 to 16 inches	11.06	18.9	33.79	22.68
16 to 24 inches	3.17	5.4	13.86	9.30
24 to 32 inches			7.30	4.90
32 to 40 inches			6.31	4.23
Totals	58.25	99.8	148.95	99.97

This comparison does not in itself prove that sugar cane is more deep-rooted in sandy soil than in a more compact clay soil, but in conjunction with other experience with root excavations we believe that there is such a correlation. Our feeling is that it is the factor of aeration in a sandy soil more than anything else which promotes this deep rooting; but this is merely a conjecture which may lead to other interesting experiments.

A still closer comparison is made of roots on two different soil types from two subsequent excavations on the same plantation; both excavations were of roots of irrigated H 109 of very nearly the same age and fertilizer treatment. However the comparison of the roots in the two different soil types was not free from other variables since the crop in the sandy loam was second-ratoon cane while the crop in the semi-adobe was plant cane. The results are given in table VI.

TABLE VI

QUANTITIES AND DISTRIBUTION OF THE ROOTS OF 21-MONTHS-OLD SECOND RATOONS IN A LOOSE ALLUVIAL SILT AS COMPARED WITH 22-MONTHS-OLD PLANT CANE IN A SEMI-ADOBE, VARIETY H109

DEPTH IN THE SOIL	22-MONTHS-OLD H-109 IN SEMI-ADOBE SOIL		21-MONTHS-OLD H109 IN LOOSE ALLUVIAL SILT	
	Grams	Per cent.	Grams	Per cent.
Hilled-up soil between the rows	9.58	5.43	3.57	2.20
0 to 2 inches	47.66	27.00	28.94	17.86
2 to 8 inches	93.02	52.70	68.09	42.03
8 to 16 inches	19.36	10.97	38.04	23.48
16 to 24 inches	4.36	2.49	14.38	8.87
24 to 32 inches	1.92	1.08	7.18	4.43
32 to 40 inches	.58	.03	1.76	.98
Totals	176.48	99.70	161.96	99.85

The cane in the loose alluvial silt had a notably larger proportion of roots in the lower levels of soil than the cane in the semi-adobe, yet even in the loose alluvial silt 62 per cent. of the roots were above the 8-inch level. Notwithstanding the fact that the cane in loose alluvial soil was a ratoon crop, and the cane in the semi-adobe was a plant crop, our wide experience in these root studies leads us to feel that of the two variables in this comparison the difference in soil character was the more important one. A diagrammatic drawing of the roots of the 21-months-old H 109 ratoons, the weights of which are recorded in table VI, is shown in fig. 4. An interesting point is that in every excavation to date the cane tonnage of the aerial parts has correlated with the root weights. In the above comparison the 176 grams of roots from the semi-adobe soil was associated with 115 tons of cane per acre, while the 161 grams of roots from the loose alluvial silt was associated with 113 tons of cane per acre.

The next excavations undertaken were of three-months-old cane of two different varieties growing under entirely comparable conditions. The results are given in table VII.

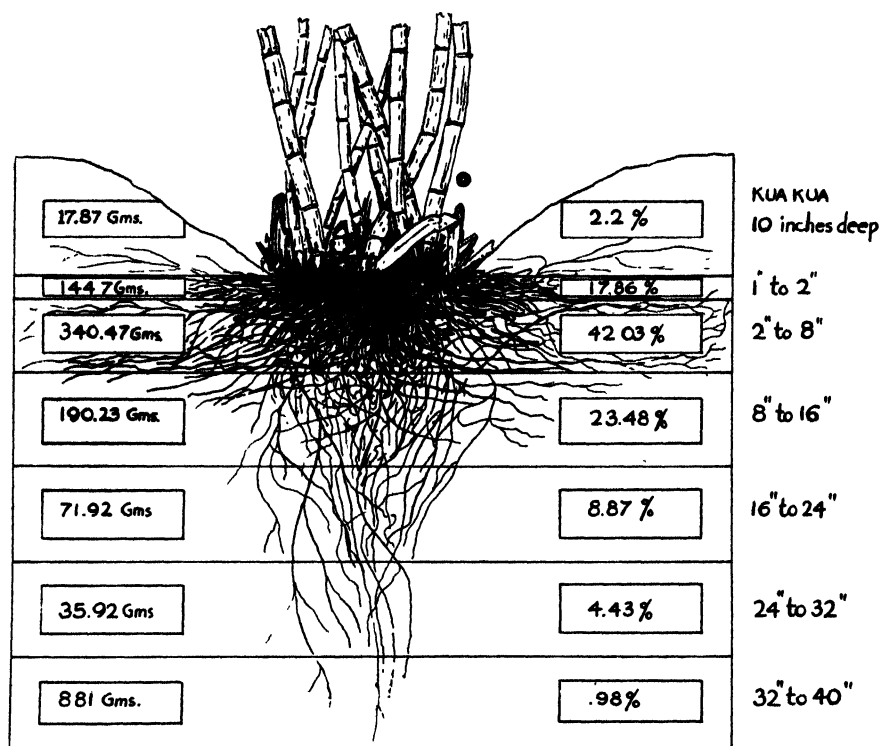


FIG. 4. A diagrammatic drawing prepared from the root weights of 21-months-old H109 sugarcane recorded in table VI. The roots in the lowest levels of the excavations are always found in the center of the pit immediately underneath the aerial parts of the cane plant, giving the root mass something of the shape of a turnip. Drawing by JAMES WILDER and TWIGG SMITH.

TABLE VII

COMPARISON OF THE ROOT QUANTITIES AND DISTRIBUTION OF 3-MONTHS-OLD LAHAINA AND H109 VARIETIES UNDER COMPARABLE CONDITIONS OF ENVIRONMENT

DEPTH IN THE SOIL	3-MONTHS-OLD LAHAINA VARIETY		3-MONTHS-OLD H109 VARIETY	
	Grams	Per cent.	Grams	Per cent.
Topmost 8 inches	13.51	87.86	28.03	88.51
8 to 16 inches	1.74	11.32	3.21	10.13
16 to 24 inches.....	0.12	.80	0.42	1.35
Totals	15.37	99.98	31.66	99.99

These excavation results show the greater root quantity of the H 109 variety as compared to the Lahaina variety, and also the higher center of gravity in the roots of young cane as compared to cane of greater age. It is noteworthy that although the total roots of the H 109 exceed the total roots of the Lahaina by more than 100 per cent., the percentage distribution of the roots in the different levels of the soil is very nearly identical for the two varieties. As regards the differences in root quantities between the two varieties, Lahaina cane is notably susceptible to several different types of root failure; and the small root quantities tabulated here are probably the result of one of the growth-failure agencies.

Another comparison between the root quantities of the Lahaina and H 109 varieties was possible in cane of nearly the same age, under comparable environmental conditions, and the results are shown in table VIII.

TABLE VIII

COMPARISON OF THE ROOT QUANTITIES AND DISTRIBUTION OF 16-MONTHS-OLD LAHAINA CANE AND 15-MONTHS-OLD H109 UNDER COMPARABLE CONDITIONS OF ENVIRONMENT;
BOTH VARIETIES WERE RATOON CROPS, GROWING ON THE RIDGES
RATHER THAN IN THE FURROWS

DEPTH IN THE SOIL	16-MONTHS-OLD LAHAINA RATOONS		15-MONTHS-OLD H109 RATOONS	
	Grams	Per cent.	Grams	Per cent.
0 to 8 inches	70.85	19.09	48.14	12.86
8 to 16 inches	159.85	43.08	176.90	47.24
16 to 24 inches	86.97	23.42	94.98	25.36
24 to 32 inches	41.38	11.15	43.48	11.61
32 to 40 inches	12.05	3.24	10.96	2.92
Totals	371.10	99.98	374.46	99.99

These results show no appreciable differences in either root quantities or distribution for these two varieties. The large quantities of roots per stool is due to the fact that in the fields where these studies were made, the stools were much farther apart than is usually the case in most fields. Figured out on the basis of roots per volume of soil the quantities of roots in the studies recorded in table VIII were not unusual. In the Hawaiian Islands there are two distinct types of practice in raising sugar cane; one practice is to grow the cane in furrows and the other is to hill up the cane so that it is growing on the ridges. In the case of the cane for which the root studies are recorded in table VIII, the practice had been to hill up the cane in contrast to the cane in all previous studies where the plants had been growing in the furrows. The results from the hilled-up cane are markedly different



FIG. 5. Root masses of 16-months-old cane of the Lahaina variety at different levels in depth in the soil. The root masses from left to right are collections from the 8 inch soil levels in sequence from the topmost stratum downwards. The outstanding variable influencing the root distribution of the cane in this particular study was the hilling-up of this cane while the cane whose roots are shown in fig. 3 was growing in furrows.

from the root results from the cane in furrows; whereas in the cane in furrows the largest proportion of the roots was found in the topmost eight inches, in the hilled-up cane the largest proportion was found in the level from eight to sixteen inches in depth. A photograph showing graphically the root masses of the 16-months-old Lahaina cane at the different levels in the soil is reproduced in fig. 5. In the hilled-up cane, however, as in the cane in furrows the quantity of roots per volume of soil was greatest in the topmost eight inches. A diagrammatic drawing of the root distribution of the hilled-up H 109 cane, the root weights of which are recorded in table VIII, is reproduced in fig. 6. In both methods of cultivation, excavation studies to date have shown in all cases more than 85 per cent. of the roots existing above the 24-inch level and a large part of the results have shown even higher proportions of the roots in the topmost two feet.

There was one further comparison made of root quantities and distribution, the results of which are seen in table IX. Both varieties in this comparison were growing in a loose sandy soil.

The variety D 1135 is often grown under unirrigated conditions and is generally regarded as more drouth resistant than most sugar cane varieties. In such a connection it is interesting to note that in the foregoing excavations not only are larger proportions of the D 1135 roots in the deeper levels of the soil but there is also a much greater total quantity. The subject of the ratio of roots to aerial parts in connection with drouth resistance in sugar cane will be developed in a subsequent paper. In the foregoing excavation studies as in those previous, more than 85 per cent. of the total roots were in the topmost 24 inches of soil. With the results recorded in this paper from the sixteen different excavations under extremely diverse conditions of environment, each result being the average of five plants,

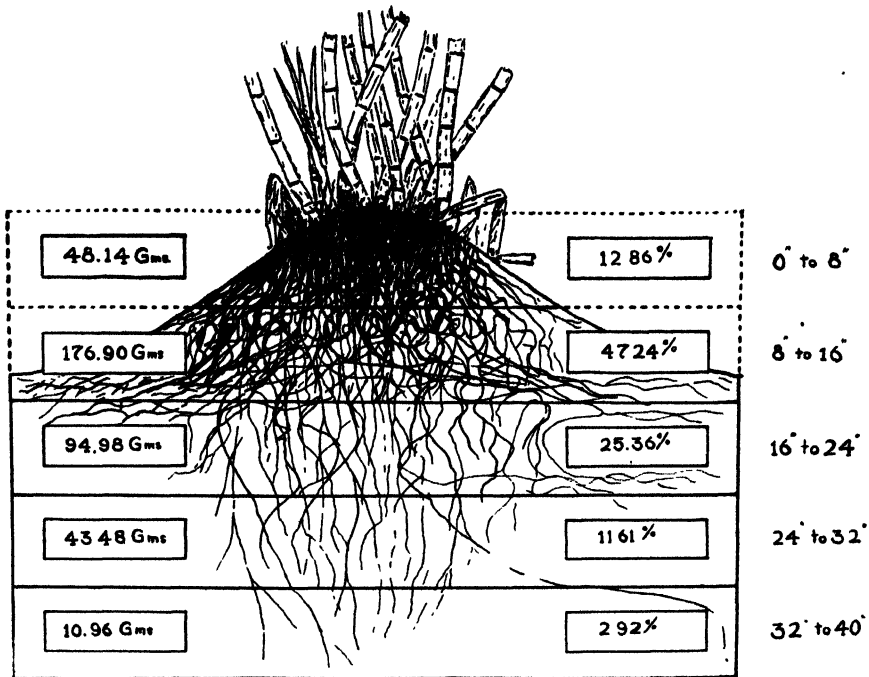


FIG. 6. Diagrammatic drawing by JAMES WILDER made from the root weights of the sugar cane variety H109 recorded in table VIII. This cane was hilled-up as contrasted with the cane illustrated in fig. 4 which was growing in furrows.

TABLE IX

COMPARISON OF THE QUALITIES AND DISTRIBUTION OF THE ROOTS OF THE D1135 AND H109 VARIETIES; BOTH VARIETIES WERE 12-MONTHS-OLD AND GROWN UNDER COMPARABLE CONDITIONS

DEPTH IN THE SOIL	D1135		H109	
	Grams	Per cent.	Grams	Per cent.
Hilled-up soil between the rows	18.83	10.25	4.44	3.65
0 to 8 inches	89.73	48.85	72.62	59.81
8 to 16 inches	34.02	18.52	29.54	24.33
16 to 24 inches	21.63	11.77	5.15	4.24
24 to 32 inches	13.14	7.15	5.44	4.48
32 to 40 inches	6.30	3.43	4.22	3.47
Totals	183.65	99.97	121.41	99.98

there is recorded a sufficient mass of evidence to indicate that a large proportion of the roots of sugar cane under Hawaiian conditions exist in the

uppermost levels of soil. In none of the foregoing studies have we found as high as 15 per cent. of the roots below the 24-inch level. These methods and results have led to other modes of attack and lines of experimentation which will be presented in subsequent publications.

Some applications of the results

It will be obvious to investigators of the soil environment of sugar cane that soil samples for chemical or physical analyses, to have the greatest application, will be from the layers in depth where the greatest proportion of roots occur. It seems safe to disregard sampling below the 48-inch level at the most, and many investigators will feel that in most cases samples will yield the most valuable information if taken from the topmost eight inches, and from the level 16 to 24 inches in depth. Samples for soil moisture will also be of the greatest value from those strata.

Plantation men have already suggested that these results may indicate that the same quantity of irrigation water applied in several frequent applications would be of greater value than a single application in an effort to secure deep penetration of the water. If one studies carefully the diagrammatic drawing of the roots of the cane in furrows, shown in fig. 4, in comparison with the drawing of the roots of the hilled-up cane, shown in fig. 6, it is apparent that irrigation water, and soluble fertilizers applied in the irrigation water, will reach a much larger proportion of the roots of cane in furrows than of the hilled-up cane where the irrigation water runs between the rows.

On the other hand, if one is growing a cane variety susceptible to root rots or if the cane is to be unirrigated, the roots of the cane on the ridges will secure much better aeration and develop greater feeding surfaces than the roots of the cane in furrows, where the blanket of soil between the rows shuts off aeration to some extent.

In excavating the roots in the foregoing studies it has been observed in many instances that the root masses from the topmost layers will be a considerably lighter color, a light brown, than the root masses from the lower levels of soil which are dead black. This difference in color seems to be due to the much greater number of small secondary feeding roots in the root masses from the topmost layers than in the lower layers of soil. It is also observable that there is a considerably greater amount of cortex rots on the roots from the lower levels of soil than on the roots from near the surface. These observations have led to the feeling, admittedly unsupported by quantitative data, that aeration is the chief factor in developing secondary feeding roots and in preventing cortex and probably stele rots.

The quantitative data presented in the foregoing tables indicates the necessity of shallow cultivation to avoid cutting the roots. Mr. FRANK BROADBENT, Agriculturist of the Hawaiian Commercial and Sugar Company, has pointed out the heavy exudation of the plant juices from the cut end of cane roots and this has since been observed very commonly in these excavation studies. On the other hand one feels that if aeration is the chief factor in promoting the maintenance of good feeding surfaces on the roots, then deep tillage preparatory to planting, and the incorporation of organic matter in the lower levels of the soil, are extremely important.

Sugar cane in the Hawaiian Islands is grown with almost no rotation of the fields to other crops and with but slightly more fallowing. This has led to the question as to how long the crop may be continued in the absence of such practices without injury to the yields. The frequent answer to this has been that the roots of the cane crops provide a quantity of organic matter which remains in the soil and so lessens the need for rotation and fallowing. There have been no quantitative data of the amounts of organic matter returned to the soil by such roots, so that the question has admittedly been an open one. Based on 8,500 stools of cane per acre, found on most irrigated plantations in Hawaii, the root quantities found per stool and reported upon in this paper mean that from 0.9 to 1.85 of a ton of dry organic matter is returned to the soil by the cane roots per crop. The writer would not attempt to say whether this is sufficient to perpetuate the cane crop, but the data at least give a definite basis for discussion and experimentation.

Summary

1. A method was developed for growing sugar cane in boxes which had detachable sides and horizontally placed wire screens; the soil could be washed from these boxes with a stream of water, leaving the roots exposed and held in their natural position by the screens of wire netting. By cutting the roots at given levels, washing them, and weighing them oven-dried, a quantitative knowledge of the distribution of the roots was available. This method was superseded by a method for studying roots under field conditions, but the box method is still useful to supplement the field method.

2. A method of studying the distribution of plants growing under field conditions was developed from the root-study-box method. An excavation was made to the middle points between the cane rows on either side of the plants being studied and to a depth which was made, for convenience, eight inches. As the soil was excavated it was thrown on a wire screen of quarter-inch mesh and as the soil was sifted through the screen the cane roots from the excavated area were separated out and collected in bags.

When the excavation of the first eight inches was completed the excavation was pushed eight inches deeper and the roots from that area collected separately. This collection of roots was continued to lower levels in the soil until only negligible quantities of roots were secured. The roots of the plants being studied which extended beyond the sides of the excavation were compensated by the roots of the plants in the rows adjoining, so that the error from that source was small.

The roots collected from the different levels in depth in the soil were washed, oven-dried and weighed, and the weights of the roots seem to be as good an expression of root surfaces as linear measurements which are much more difficult to secure. The roots of five plants were excavated at a time and the results averaged per plant.

3. Sixteen excavations of five plants each under very divergent conditions of environment are reported in this paper. In the cane growing in furrows a very large proportion of the roots was found in the topmost eight inches of the soil; in every case studied more than 58 per cent. of the roots have been above the eight-inch level in the soil. In the hilled-up cane the largest proportion of the roots, in the cases studied to date, was found in the level from eight to sixteen inches in depth in the soil. In cane, either hilled-up or in furrows, more than 85 per cent. of the roots were found in the topmost 24 inches.

4. The color of the root masses from the uppermost levels in the soil was observed in many cases to be light brown, while that of the root masses from the lower levels in the soil was a darker brown or even black. This led to the observation that there were many more secondary roots with actively feeding surfaces in the root masses from the upper levels of soil than in the masses from greater depths in the soil and these secondary roots gave the lighter brown color to root masses near the soil surface. Rots of the root cortex were also more general in the lower depths of the soil and these rots gave a darker color to the root masses from deep in the soil.

5. A few applications of the knowledge gained in these studies are mentioned.

EXPERIMENT STATION, HAWAIIAN SUGAR PLANTERS' ASSOCIATION,
HONOLULU, HAWAII

LITERATURE CITED

1. VENKATRAMAN, T. S., and THOMAS, R. Simple contrivances for studying root development in agricultural crops. *Agr. Jour. of India* 19: 509. 1924.
2. WEAVER, JOHN E., JEAN, FRANK C., CRIST, JOHN W. Development and activities of roots of crop plants. Carnegie Institution of Washington, Publ. no. 316. 1922.
3. WEAVER, JOHN E. Root development of field crops. McGraw-Hill Book Co., New York. 1926.

THE DIGESTION OF PECTIN AND METHYLATED GLUCoses BY VARIOUS ORGANISMS*

HAROLD W. COLES

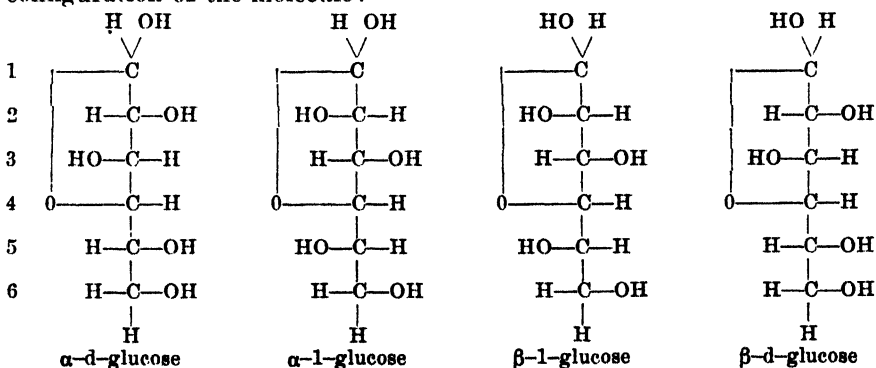
Introduction

This preliminary paper is the result of work started with the object of determining the effect of introduced methyl groups upon the digestion of carbohydrates by microorganisms. At the same time, it was hoped that methylated glucoses and pectin might offer a means of distinguishing between *Bact. aertrycke*, *Bact. schotmüller*, and other closely related forms. These two organisms, up to the present time, are not distinguishable on the basis of morphological, cultural, or physiological characteristics. It was also thought that possibly a relation might exist between the digestion of pectin and the methylated glucoses.

The only paper in the literature recording the digestion of methylated hexoses by organisms is that of IRVINE and HOGG (4) who record a few experiments made with 3-monomethyl glucose. It was found by these authors that living top yeast and macerated extract, prepared from dried Munich bottom yeast, had no action upon the sugar. They also found that, of the seven species of bacteria tried, *Bacterium coli commune*, *Bact. lactis aerogenes*, *Bact. proteus*, *Bact. paratyphosum*, *Bact. cloacae*, *Bact. typhosum*, and *Staphylococcus pyogenes aureus*, all of which are glucose fermenters, only *Bact. cloacae* Jordan digested the methylated glucose with the production of acid and gas.

Methylated glucoses

Glucose may have the following structures¹ depending upon the spacial configuration of the molecule:



* Reported at the meeting of the Iowa Academy of Sciences, April, 1926.

¹ The recent paper of HIRST, Jour. Chem. Soc., London 129: 350. 1926, indicates that the oxide linkage of glucose should be amylenic <1, 5> instead of butylenic <1, 4> as shown.

It is seen that we might theoretically have methoxy groups attached to carbons one to six on each of the configurations given. Furthermore, we might have all possible combinations of two to five methyl groups for each configuration, and if we include the so-called γ -sugars (compounds in which the oxidic linkage is believed to be other than butylene oxidic $<1, 4>$) the list is greatly increased.

A simple calculation will show, then, that we have theoretically possible the following methylated glucoses: 20 monomethyl glucoses (including the methyl glucosides), 56 dimethyl glucoses, 76 trimethyl glucoses, 52 tetramethyl glucoses, and 20 pentamethyl glucoses, a total of 224 different methylated glucoses, not including the gamma sugars, since each glucose derivative can occur as the α - or β -modification and each of these as dextro- or levo-glucose.

A careful search of the literature revealed that only a very few of these theoretically possible sugars have been prepared. These are tabulated below, the doubtful ones being indicated by a question mark.

TABLE I

SUMMARY OF METHYLATED GLUCOSES DESCRIBED IN THE LITERATURE

NUMBER OF METHYL GROUPS				
1	2	3	4	5
α -d-1	α -d-1, 3	α -d-1, 2, 3,	α -d-1, 2, 3, 5	α -d-1, 2, 3 5, 6
β -d-1	α -d-2, 3	α -d-1, 2, 5, (?)	β -d-1, 2, 3, 5	β -d-1, 2, 3 5, 6
α -d-2 (?)	β -d-2, 3	α -d-2, 3, 5,	α -d-1, 2, 3, 6	
α -d-3	α -d-2, 5 (?)	α -d-2, 3, 6,	β -d-1, 2, 3, 6	
β -d-3		α -d-3, 5, 6,	α -d-1, 4, 5, 6 (?)	
α -d-6 (?)		α -d-2, 5, 6, (?)	β -d-2, 3, 5, 6	
			α -d-2, 3, 5, 6	
			α -d-3, 4, 5, 6	

Materials and methods

The media used in these investigations were of two kinds: One containing peptone, and the other containing no peptone, and hence no carbon except the carbon of the pectin or the carbon of the methylated glucoses. They were made up as follows:

MEDIUM WITH PEPTONE

2 gms. bacto-peptone
 1 gm. K_2HPO_4
 10 cc. 1 per cent. Andrades indicator
 3 gms. pectin or sugar
 1000 cc. distilled water

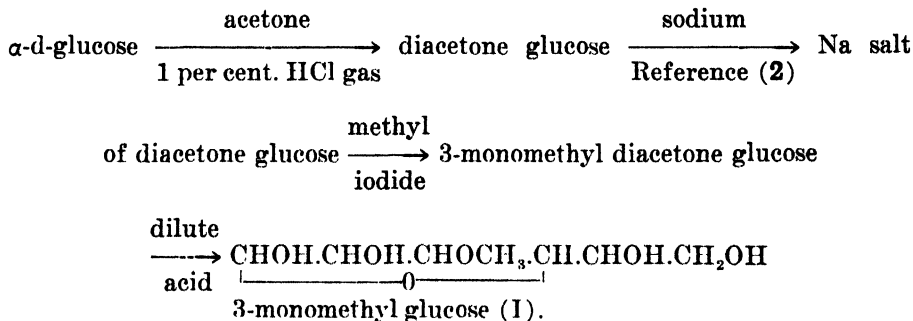
MEDIUM WITHOUT PEPTONE

2 gms. ammonium chloride
 2 gms. K_2HPO_4
 10 cc. 1 per cent. Andrades indicator
 3 gms. pectin or sugar
 1000 cc. distilled water

The media were adjusted to a P_H of 7.0 to 7.2 and tubed. In all of the work, twenty-four hour glucose-phosphate agar slants were used for inoculation of the culture media. Inoculations in every case were made as heavy as possible, care being especially taken with the medium containing no peptone to see that the inoculations were positive. The tubes, after inoculation, were incubated 24 to 98 hours at 37°C ., excepting those organisms whose optimum was 25° to 27°C ., and any acid, gas or gumminess recorded each day.

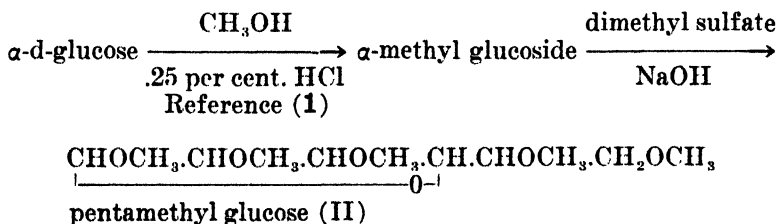
The pectin, a very pure form, was obtained from the Research Laboratories of the California Fruit Growers Exchange, and was used as received. It contained, as reported by them, aluminium oxide as an impurity. It was tested for reducing sugar which was found to be absent.

The method followed for the preparation of the 3-monomethyl glucose was essentially that of IRVINE and HOGG (4). The reactions involved are:

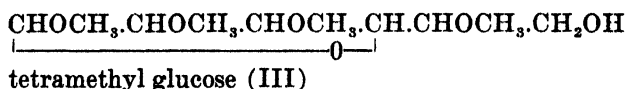


The 3-monomethyl glucose thus secured is readily soluble in water, less so in methyl alcohol, and is very sparingly soluble in other organic solvents. It has a melting point of $157\text{--}158^\circ \text{C}$., and a rotation in water of $+55.5^\circ$ (final). It exhibits mutarotation. It was purified by recrystallization from methyl alcohol.

The preparation of 1, 2, 3, 5-tetramethyl glucose and 1, 2, 3, 5, 6-pentamethyl glucose may be described together, since they are formed in the same methylation process. The method of HAWORTH (3) was used. The reaction occurs as follows:



and



The methylated glucoses thus secured are syrups. They were repeatedly fractionated for purification. They did not reduce Fehling's solution.

The tetramethyl glucose (III) is soluble in water, chloroform, methyl iodide and in common organic solvents. It has a refractive index number

equal to 1.4583 and $D_{\frac{20}{4}}$ equal to 1.158. It does not show mutarotation.

The pentamethyl glucose (II) is soluble in alcohol, water, acetone, ether, and methyl iodide. Index of refraction, n_D is equal to 1.4454. It has the following rotations: +147.4° in water and +153.9° in alcohol. It does not show mutarotation.

Description of organisms

The organisms of group I belong to the colon-typhoid group and were mainly of fecal origin. Those of group II were obtained from activated sludge from creamery wastes, and group III are organisms from miscellaneous sources. The figures in parentheses indicate the number of strains used. All cultures which attacked pectin and the methylated glucoses were examined morphologically and culturally to determine their position in the classification of the colon-typhoid group as given by WELDIN and LEVINE (5).

GROUP I

Bacterium coli (4), *Bact. paragrünthali* (2), *Bact. communior* (1), *Bact. coscoroba* (2), *Bact. grünthali* (5), *Bact. neapolitanum* (1), *Bact. pseudocoloides* (1), *Bact. schafferi* (4), *Bact. vesiculosum* (2), *Bact. aerogenes* (9), *Bact. cloacae* (7), *Bact. levans* (3), *Bact. oxytocum* (13), *Bact. vulgaris* (6), *Bact. abortivoequinum* (2), *Bact. aertrycke* (1), *Bact. paratyphi* (5), *Bact. flexneri* (6), *Bact. schotmülleri* (4), *Bact. pullorum* (5), *Bact. morgani* (2), *Bact. enteritidis* (3), *Bact. suipestifer* (3), *Bact. ambiguum* (1), *Bact. typhi murium* (1), *Bact. dispar* (1), *Bact. typhi* (2), *Bact. alkalescens* (1), *Bact. sanguinarium* (2), *Bact. anatum* (1), *Bact. pfaffii* (1), *Bact. jeffersonii* (1), *Bact. rettgeri* (1), *Bact. shigae* (1), *Bact. rhinoscleromatis* (1), *Bact. viscosum* (1), *Bact. viscosum aerogenes* (1).

GROUP II

Bacillus albolactus (2), *B. cereus* (4), *B. mesentericus* (1), *B. panis* (2), *B. megatherium* (1), *B. atterimus* (1), *B. niger* (1), *Pseudomonas myxo-*

genes (1), *Pseud. fluorescens* (1), *Pseud. ovalis* (2), *Pseud. pavonacea* (3), *Flavobacterium ovalis* (2), *Flav. acetylicum* (2), *Flav. suaveolens* (3), *Flav. zettnowi* (1), *Flav. deceduosum* (1), *Serratia rubida* (2), *Ser. rubrica* (2), *Ser. amylocluber* (1), *Bact. metalkalescens* (1), *Rhodococcus corallinus* (1).

GROUP III

Saccharomyces ellipsoideus (2), *Sac. cerevisiae* (1), *Torula rosea* (1), *B. subtilis* (2), *B. sphaericus* (1), *B. mesentericus* (1), *Staphylococcus aureus* (2), *Staph. albus* (3), *Ser. marcescens* (3), *B. fusiformis* (1), *Pasteurella cholera gallivarum* (1), *B. aceto-ethylicus* (2), *Pseud. cyanogenes* (3), *Pseud. pyocyanea* (2), *Pseud. fluorescens* (1), *Sac. pombe* (1), *Past. suisepitica* (1), *Sarcina lutea* (1), *Monilia variabilis* (1), *Micrococcus luteus* (1), *B. mesentericus fuscus* (1), *Bact. abortus* (1), *Micr. flavescens* (1).

Results

The cultures which attacked the pectin with the production of acid and gas are the following, the numbers in parentheses being the laboratory numbers of the cultures: Seven out of thirteen strains of *Bact. oxytocum* (305, 369, 499, 139, 261, 269, 270); four out of nine strains of *Bact. aerogenes* (117, 80, 256, 257); two out of two strains of *Bact. viscosum aerogenes* (248, 298); and two out of two strains of *Bact. aceto-ethylicus* (172, 173). Garden soil and manure produced acid and no gas, and garden soil produced gumminess in both media. *Bact. schafferi* (103) produced gumminess in peptone media. All other cultures of Groups 1 to 3 failed to attack pectin with the production of acid, gas or gumminess. There was no difference between the two media, i.e., no culture produced acid or gas in the synthetic non-peptone medium which did not produce it in the peptone medium, and vice versa.

A larger variety of cultures produced acid and gas from the 3-monomethyl glucose. They were: Six out of seven strains of *Bact. cloacae* (233, 235, 236, 264, 136, 263); one out of three strains of *Bact. levans* (238); one out of fourteen strains of *Bact. oxytocum* (499); one strain of *Bact. pseudocoloides* (486); and one out of nine strains of *Bact. aerogenes* (268). Manure gave acid and gas, but garden soil gave acid and no gas.

The tetramethyl and pentamethyl glucoses were not digested by any of the cultures of Groups 1, 2 or 3 with the production of acid and gas. The test with manure was indefinite, a slight acidity being produced with a bubble of gas.

Discussion

From a consideration of the cultures used and the cultures digesting pectin with the production of acid and gas, it is evident that only those

organisms commonly occurring in the soil are capable of attacking pectin with the production of acid and gas. None of the intestinal forms, classified in the sub-genus *Escherichia* of the genus *Bacterium*, produced acid or gas from the pectin. It was to be expected that organisms digesting pectin would be found in the soil, since the rotting of wood and plant tissue, containing pectin bodies, takes place readily in the soil.

Again, it is evident that only those organisms occurring commonly in the soil are capable of attacking 3-monomethyl-glucose with the production of acid and gas. It was expected that very few organisms, if any, would be able to touch the tetramethyl and pentamethyl glucoses. In these two sugars the hydroxyl groups are protected by methyl groups, and this parallels the cellulose molecule in which hydroxyl groups are presumably bound in ether linkages. It seems, therefore, that those organisms of the colon-typhoid group, which are vigorous fermenters of glucose, are unable to attack methylated glucoses when the number of methyl groups becomes large.

It is interesting to speculate just how the number and position of the methyl groups in the sugar molecule affects the digesting ability of the organisms. It is hoped to make these speculations the basis of a later investigation on methylated hexoses.

Conclusions

1. Pectin and the methylated glucoses tried were not digested with the production of acid or gas by those members of the colon-typhoid group commonly found in feces.

2. The organisms attacking pectin and 3-monomethyl glucose with the production of acid and gas were those generally associated with the soil.

3. Pectin and the methylated glucoses tried were not digested with the production of acid or gas by organisms isolated from the activated sludge of creamery wastes.

4. All of the members of the colon-typhoid group tested were incapable of digesting 1, 2, 3, 5-tetramethyl glucose and 1, 2, 3, 5, 6-pentamethyl glucose.

5. *Bact. schotmülleri*, *Bact. aertrycke* and other closely related forms can not be differentiated on the basis of the digestion of pectin and the methylated glucoses used.

6. It is suggested that an agar medium containing only 3-monomethyl glucose may be useful for the isolation of *Bact. cloacae*.

The helpful suggestions of Dr. R. M. HIXON of the Chemistry Department and Dr. MAX LEVINE of the Bacteriology Department are gratefully acknowledged.

LABORATORIES OF PLANT CHEMISTRY AND BACTERIOLOGY,
IOWA STATE COLLEGE,
AMES, IOWA

LITERATURE CITED

1. BOURQUELOT, E. La synthese biochimique des d-glucosides d'alcools monovalents. II. Alcool-d-glucosides- α . *Ann. de Chim. (Paris)* **3**: 287-337. 1915.
2. FREUDENBERG, K., and HIXON, R. M. Zur Kenntniss der Aceton-Zucker IV: Versuche mit Galaktose und Mannose. *Ber. deut. chem. Gesell.* **56**: 2119-2128. 1923.
3. HAWORTH, W. N. A new method of preparing alkylated sugars. *Jour. Chem. Soc. (London)* **107**: 8-16. 1915.
4. IRVINE, J. C., and HOGG, T. P. Partially methylated glucoses. Part III. Monomethyl glucose. *Jour. Chem. Soc. (London)* **105**: 1386-1396. 1914.
5. WELDIN, J. C., and LEVINE, MAX. An artificial key to the species and varieties of the colon-typhoid or intestinal group of bacilli. *Proc. Amer. Soc. of Bact., Bact. Abst.* **7**: 13-15. 1923.

GROWTH OF YOUNG WHEAT PLANTS IN AUTO-IRRIGATED SOILS, AS RELATED TO THE WATER-SUPPLYING POWER OF THE SOIL AND TO THE ADJUSTMENT OF THE AUTO-IRRIGATOR

BURTON E. LIVINGSTON, TAKEWO HEMMI AND J. DEAN WILSON

(WITH ONE FIGURE)

Introduction

The auto-irrigator (4, 5) furnishes a convenient means for maintaining approximately constant moisture contents in the soil masses of pot cultures, the maintained moisture content being dependent on the kind of soil used (indicated by its water-holding power, moisture equivalent, etc.), on the packing of the soil, and on the hydrostatic head against which water moves from the irrigator reservoir into the soil mass.

The experiments briefly described below were planned as a preliminary study of the relations between the growth of young wheat plants in auto-irrigated pot cultures and the water-supplying power of the soil about their roots, this dynamic soil feature being itself controlled, for each kind of soil used, by the water content of the soil, controlled in turn by the height of the mercury column introduced between the irrigator reservoir and the water-supplying, porous-porcelain cone. The experiment here reported was carried out by HEMMI in the greenhouse of the Laboratory of Plant Physiology of the Johns Hopkins University, in the winter of 1922-23, and he left his notes with the other authors of this paper when he returned to Japan. The results seem worthy of publication because of the great fundamental importance of the water-supplying power of the soil in plant physiology and plant culture, and because they add considerably to our knowledge of the manner in which the auto-irrigator operates in practice. It seems to be becoming more generally appreciated that the influential conditions of plant environments must be controlled or quantitatively recorded for effective experimentation in plant physiology, and the moisture conditions of the soil are now among the environmental conditions most susceptible of control and satisfactory measurement.

Materials and methods

Three different kinds of soils were used (intended to be like numbers 3, 6, and 9 of LIVINGSTON and KOKETSU's (6) series): a half-and-half sand-

loam mixture with a water-holding power of 39.2 per cent., a fertile loam with a water-holding power of 60.7 per cent., and a half-and-half humus-loam mixture with a water-holding power of 95.1 per cent., these values being determined by the Hilgard method and expressed on the basis of dry weight. On the basis of dry volume they are 53.5, 57.5, and 78.3 per cent., respectively. Tinned sheet-iron containers were used as pots, 15 cm. in diameter and 20 cm. high, each furnished with an auto-irrigator, employing the porous-porcelain cone after the manner described by LIVINGSTON in 1918. Between the reservoir and the cone was inserted a glass U-tube containing mercury, to introduce hydrostatic head against which all water passing into the soil mass must move. Five containers were used for each kind of soil, with different heights of mercury column in the U-tube, these heights being 2, 10, 20, 30, and 40 cm. The irrigators were allowed to operate preliminarily until the capillary water system of the soil closely approached equilibrium with the mercury column, as shown by the fact that the soil masses ceased practically to gain in weight. Then five wheat seeds were planted in each container and the plants produced were allowed to develop six weeks. During this period the water-supplying power of the soil, at a depth of 6 centimeters, was determined from time to time by the soil point method (6, 7), the results being recorded as milligrams of water absorbed by a single soil point in a two-hour period. (Later work indicates that a one-hour period is better for such determinations, but the results here reported are satisfactory for comparisons within their own series, though they are not comparable with results based upon any other time period.) The standard soil point, of porous porcelain, has an absorbing surface of about 12 sq. cm., and the two-hour indices of water-supplying power here given may consequently be converted so as to refer to a single square centimeter of absorbing surface by dividing each value by 12. No reliable supplying-power value was secured for the soil in equilibrium with the two-centimeter column of mercury in any case, nor was a reliable value secured for loam in equilibrium with the 10 cm. column, these omissions being due to the use of a shorter time period for the soil-point tests in the cases mentioned. The values were very high in all these cases, surely above the absorbing capacity of the soil point for the soils in equilibrium with the two-centimeter column. The omissions do not detract materially from the value of the results, as will be seen later when the numerical data of the experiment are presented.

Determinations of the water content of each container were also made from time to time, by means of small samples removed with an ordinary cork borer with a diameter of about 1 cm., the holes in the soil being immediately filled with fresh soil of the same kind after each sampling. The

water-content values were recorded as percentages of the volume of the soil and also as percentages of its dry weight.

The several indices of water-supplying power for each container were finally averaged, as were also the several indices of water content. At the end of the experiment the tops of the plants were cut off at the soil surface and their green and dry weights determined, as well as their water contents, as indices of growth vigor. These plant values were recorded as averages per plant six weeks old. The auto-irrigators were read from time to time during the experiment and the total amount of water delivered to the soil of each container during the six-week period was secured by summing the readings.

Results

The data secured from the experiment are shown in table I.

The values given in the table are represented by the graphs of the accompanying figure 1, there being a graph for each of the three kinds of soil.

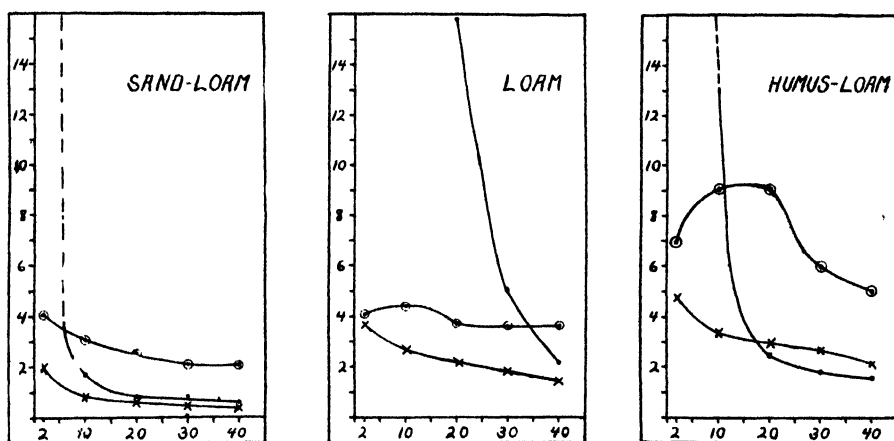


FIG. 1. Graphs of water-supplying power, water content, and plant growth (green weight of tops of six-week wheat plants), as these were related to the mercury column in the auto-irrigator with which the soil was in equilibrium. Three different soils are represented. The steepest curve is for water-supplying power, the curve for water content has crosses at the points, and that for green weight has circles at the points. Abscissas are heights of mercury column in centimeters. Data are from accompanying table.

Abscissas represent heights of the mercury columns, and ordinates represent supplying-power values (for the steepest curve), water content values (for the lowest curve, marked with crosses at the points), and green weights of tops (for the intermediate curve, marked with circles at the points).

TABLE I

WATER-SUPPLYING POWER AND WATER CONTENT OF AUTO-IRRIGATED SOILS IN EQUILIBRIUM WITH DIFFERENT HEIGHTS OF MERCURY COLUMN, TOGETHER WITH GREEN AND DRY WEIGHTS AND WATER CONTENTS OF TOPS OF WHEAT PLANTS SIX WEEKS OLD THAT HAD GROWN IN THESE SOILS

KIND OF SOIL	TOTAL AMOUNT OF WATER DELIVERED TO SOIL BY IRRIGATOR IN 6 WEEKS	HEIGHT OF MERCURY COLUMN IN IRRIGATOR	AVERAGE INDEX OF WATER-SUPPLYING POWER FOR 2-HOUR PERIOD OF EXPOSURE	AVERAGE WATER CONTENT OF SOIL		INDICES OF PLANT GROWTH		
				VOLU-METRIC	GRAVIMETRIC (ON DRY-WEIGHT BASIS)	AVERAGE GREEN WEIGHT OF TOPS PER PLANT	AVERAGE DRY WEIGHT OF TOPS PER PLANT	AVERAGE WATER CONTENT PER PLANT
	cc.	cm.	mg.	Per cent.	Per cent.	mg.	mg.	mg.
Sand-loam mixture	1421	2	20.2	14.2	404	54	350
	643	10	170	7.9	5.3	304	42	262
	347	20	90	6.2	4.2	254	36	218
	191	30	90	4.8	3.3	210	30	180
	161	40	60	4.1	2.8	210	30	180
Loam	1238	2	36.1	28.4	406	46	360
	1294	10	26.8	19.9	441	50	391
	1148	20	1580	22.4	16.0	370	44	326
	940	30	510	17.5	12.5	358	48	310
	740	40	220	14.4	10.6	360	48	312
Humus-loam mixture	1031	2	47.3	56.2	690	82	608
	610	10	1310	33.5	36.1	906	92	814
	473	20	240	29.4	32.1	904	96	808
	268	30	180	26.9	29.4	598	66	532
	225	40	160	21.4	23.3	504	58	446

Discussion

The following observations may be made here, from the data. The water-supplying power consistently follows the water content in the case of each soil, though these two properties are not proportional. For each soil the water content is determined, within limits, by the height of the mercury column. The water content on an auto-irrigated soil mass may have any value between that which is in equilibrium with the highest mercury column that can be used and that corresponding to the maximum capillary content, but its lower limit is fixed by the physical characteristics of the soil employed. For any soil there is a certain range of water contents and corresponding water-supplying powers that can be established and maintained by the auto-irrigator. The upper limit of this range is the maximum capillary content, with the mercury column just balancing the water column between irrigator cone and reservoir. The lower limit is very low for sandy soils, but high for clays and humus soils, with the highest mercury column that can be applied. For the five heights of mercury column used in this experiment the ranges of water content secured were: from 20 to 5 per cent. for the sand-loam mixture, from 36 to 14 per cent. for the loam soil, and from 47 to 21 per cent. for the humus-loam mixture. The corresponding water-supplying powers ranged from very high values (probably well above 2,500) to 60 for the sand-loam mixture, to 220 for the loam soil, and to 160 for the humus-loam mixture. To secure still lower water contents and supplying-powers, it is at once suggested that still higher mercury columns be employed, but the graphs indicate that increased height of mercury column could have very little effect for the sand-loam or humus-loam mixtures, though a higher mercury column would probably lower the values in question considerably for the loam itself. The present set-up of the auto-irrigator is restricted by the fact that mercury columns higher than about 60 or 65 cm. are not practicable.

If adequate liquid strain (1, 8) might be developed in the irrigator system the resistance offered to the entrance of water into the soil might be correspondingly increased, but this has not yet been accomplished. Of course a nearly complete atmosphere of opposing pressure might be developed by the use of suction applied in the irrigator reservoir by means of an air pump, but this has not been tried. At best it could generally give resistances corresponding to no more than about 75 cm. of mercury column.

For any height of mercury column in the auto-irrigator the water-supplying power and the water content of the controlled soil were, in general, lowest for the sand-loam mixture and highest for the humus-loam mixture, being intermediate for loam. They are obviously related to the internal surface forces of the several soils, represented by their water-

holding powers. The higher the water-holding power the greater should be the height of the mercury column in the irrigator, to develop and maintain a given water-supplying power in the soil.

With few slight exceptions, the three different growth indices are consistent and consequently only those for green weight of tops are presented in the graphs. For any height of the mercury column the growth of the plants was most vigorous in the humus-loam mixture, least vigorous in the sand-loam mixture, and intermediate in loam. It is notable that the growth values corresponding to the two-centimeter column of mercury are nearly alike for the sand-loam mixture (404) and for loam (406), while the corresponding value for the humus-loam mixture (690) is much greater.

The greatest growth values for the sand-loam mixture were secured with the highest water-supplying power (not measured), with a volumetric water content of 20.2 per cent. and with the shortest mercury column (2 cm). The twenty-centimeter, thirty-centimeter, and forty-centimeter columns of mercury with the sand-loam mixture were nearly alike as to water-supplying power, water content, and growth index; and all these values were very low. With these mercury columns growth was markedly retarded, but the plants did not succumb, even with the highest mercury column. Growth would probably have ceased altogether with the highest column and the plants would probably have died, if they had been subjected to somewhat more intense conditions of evaporation and sunshine, or even if the cultures had been continued longer, since the water requirement of a plant of course increases as growth progresses, other influential conditions being unchanged.

For the loam soil the highest growth values were secured with the ten-centimeter column of mercury in the irrigator, a slight relative retardation being apparent for the two-centimeter column. This retardation is probably not to be related to water-supplying power, nor directly to water-content, but rather to the oxygen-supplying power (2, 3) of the soil, which is necessarily lower with higher water contents. With the exception of the two-centimeter mercury column, every one of the columns with loam gave very much greater growth than did any of the columns with the sand-loam mixture; the forty-centimeter column with loam gave nearly twice as much green weight per plant as did the same column with the sand-loam mixture.

A very pronounced retardation in growth is shown for the two-centimeter column with the humus-loam mixture. With this mixture the highest green-weight values were secured (being about alike) with the ten-centimeter and twenty-centimeter columns, and these growth values are by far the highest of the whole experiment. A water-supplying power of 1,310 gave the highest growth value (ten-centimeter column) but one of 240 gave a value nearly as high (twenty-centimeter column). Supplying powers

much greater than 1,300 were apparently attended by growth retardation for this soil, probably related to deficient oxygen supply, as mentioned in connection with the similar case with loam. The two-centimeter column gave nearly as poor growth as did the thirty-centimeter column. Growth was very good with the highest column, better than with any column for either of the other soils. To retard growth as much in this mixture, and in the loam soil also, as much as it was retarded in the sand-loam mixture with the forty-centimeter column of mercury, would, as is indicated above, require a still higher column and might not be attainable with any column that could be applied without actual liquid strain. Only in our most sandy soil was the lower limit of the supplying-power requirement for good growth apparently approached with any of our mercury columns.

The amounts of water delivered to the soil masses show somewhat the same relations to the heights of the mercury columns and to growth as do the water contents and water-supplying powers. These rates of delivery of water from the irrigator to the soil are of course largely dependent on the rate of evaporation from the soil surface and on the rate of water absorption by the plants.

It is of special interest and importance to call attention to the observation that the growth of the wheat plants in this experiment was clearly and profoundly influenced in some cases by some variable or variables other than the water-supplying power of the soil. With soils having very high water-supplying powers it is probable, as has been mentioned, that growth retardation is brought about by deficiency in the oxygen-supplying power of the soil. This might explain why a water-supplying power of 1,580 (loam with a twenty-centimeter mercury column) gave a green-weight value of but 370, while a water-supplying power of only 160 (humus-loam mixture with a forty-centimeter mercury column) gave a green-weight value of 504. Other considerations would need to be taken up, however, for a rational and general interpretation of such results. For example, a supplying-power of 1,310 (humus-loam mixture with a ten-centimeter mercury column) gave the highest growth value of the entire series (906 for green weight of tops), while a water-supplying power of only 240 (the same mixture with a twenty-centimeter mercury column) gave practically the same growth index (904). Other apparently anomalous cases may be found in our table.

Other chemical conditions of the soil, aside from its oxygen-supplying power, may of course have exerted influence on the growth of the plants. The loam used is known to have been a productive soil, and the two mixtures used were each half loam. It consequently does not appear likely that any of our plants were retarded by inadequacy in the supply of the necessary inorganic salts and ions. That the humus may have introduced special and

perhaps influential chemical conditions is of course possible, but no consistent interpretation of our results seems to emerge from a study of the data with this possibility in mind. It seems likely that we are here dealing with a matter of considerable complexity, one that will require much further study. It may well be that the water-absorbing powers of the root systems were different for the different treatments, with resulting differences in the ratio of water-absorbing power to transpiring power in the plants themselves. Such a suggestion leads to many possibilities, an *a priori* consideration of which would, however, be premature with our present very limited experimental data. At any rate, the problem of the water relations between plant and soil is worthy of much more serious attention than has thus far been devoted to it. The auto-irrigator, the soil point method, and the method for determining the oxygen-supplying power of the soil should all be valuable in the development of this very important portion of the field of plant physiology and physiological ecology.

In spite of the considerations suggested in the last paragraph, it remains clear that the water-supplying power of the soil was, in general, of primary importance in determining the growth of our wheat plants. If the fifteen different treatments are arranged in the descending order of green-weight values it becomes clear that the highest five growth indices are for the range of water-supplying powers from very high to 160 (humus-loam mixture), while the lowest four growth indices are for the range of water-supplying powers from 170 to 60 (sand-loam mixture).

To avoid possible partial misunderstandings or unjustified deductions, it may be mentioned that our data and interpretations refer to a Baltimore greenhouse in the winter months, to wheat plants grown for six weeks from seed, to the three specific soils used, to auto-irrigators of the type employed, and to standard soil points exposed to the soil at a depth of 6 centimeters for a period of two hours. Other plants, other treatments, and other instrumentation may be expected to give in some cases more or less different results; but the general principles here noted may be expected to obtain.

LABORATORY OF PLANT PHYSIOLOGY,
THE JOHNS HOPKINS UNIVERSITY

LITERATURE CITED

1. ASKENASY, E. Über das Saftsteigen. Verh. Naturhist. Med. Ver. Heidelberg N. F. 5: 429-448. 1897.
2. HUTCHINS, LEE M. Studies on the oxygen-supplying power of the soil as indicated by color changes in alkaline pyrogallol solution, together with quantitative observations on the oxygen-supplying

power requisite for seed germination. *Plant Physiol.* **1**: 95-150. 1926.

3. ———, and LIVINGSTON, B. E. Oxygen-supplying power of the soil as indicated by color changes in alkaline pyrogallol solution. *Jour. Agr. Res.* **25**: 133-144. 1923.
4. LIVINGSTON, B. E. Porous clay cones for the auto-irrigation of potted plants. *Plant World* **21**: 202-208. 1918.
5. ———, and HAWKINS, LON A. The water relation between plant and soil. *Carnegie Inst. Publ. no.* **204**: 1-48. 1915. Washington, D. C.
- X 6. ———, and KOKETSU, RICHIRO. The water-supplying power of the soil as related to the wilting of plants. *Soil Sci.* **9**: 469-485. 1920.
7. ———, and OHGA, ICHIRO. The summer march of soil moisture conditions on a lawn. *Ecology* **7**: 427-439. 1926.
8. URSPRUNG, A. Dritter Beitrag zur Demonstration der Flüssigkeitskohäsion. *Ber. d. bot. Ges.* **34**: 475-488. 1916.

THE CHEMICAL ANALYSIS OF PLANT TISSUES*

The work of this committee has been undertaken at the request of the executive board of the Society and in response to numerous expressions of interest from the members. In order that the purpose in view may be understood at the outset a statement of the proposed scope of activity seems to be desirable.

Plant physiologists operating in such applied fields of investigation as agronomy, horticulture and pathology have frequently experienced need for examination of their problems and results in terms of chemical composition of tissues. In many cases it is not feasible to employ a specially trained chemist for this purpose. Moreover, the earlier stages of this phase of the work call generally for the comparative results of somewhat routine chemical analyses. These may point the way to refined qualitative examination and more accurate quantitative results, for which the services of an expert will be required. There is considerable recent evidence to support the practicability of advising competent but less skilled workers in the use of general methods of chemical analysis, where comparative results are concerned. It should be recognized clearly, however, that no such recommendations as are here to be proposed can carry any guarantee of infallibility with all materials and in all situations. A reasonable exercise of critical faculties is invariably demanded of those who would attempt the execution of chemical analyses.

It is not the aim of the committee to attempt to treat completely the applications of chemistry to plant analysis. This is done already in a rather complete manner in such compilations as the *Methods of Analysis* of the Association of Official Agricultural Chemists. The methods described in this book, however, are not intended for research purposes and, like most encyclopaedic treatises, lack detailed relation to the problems of physiology. Materials of physiological interest are recognized as foundational to this report. For the present, attention will be given chiefly to the much used phases of analysis covering carbohydrates and nitrogenous constituents. In this capacity it is hoped to incorporate current improvements, add discussional approaches to the work and, in general, frame recommendations which may serve as more flexible and specialized directions than are extant in published form. In no sense is dictation of procedure to be presumed upon the experienced investigator. On the other hand, it is suggested that the committee serve as a clearing house

* Recommendations of the Committee on Methods of Chemical Analysis for the American Society of Plant Physiologists.

of information in which the contributions of experienced workers may be made available for those attempting the preliminary application of chemistry to problems of plant physiology.

A stereotyped method cannot be generally applicable to all the varying complexes of factors in different types of tissue. Perhaps the empirical methods of chemical analysis now generally used will not suffice to delineate accurately the metabolic changes of plants. Nevertheless, there remains for the present an initial span of applied chemistry to which general methods are applicable when modified to meet special requirements. The committee will endeavor to render service in this field, presenting its suggestions from time to time in this Journal. Cooperation is being extended by many workers in plant science whose contributions are herein duly acknowledged. The work will be continued in proportion to its apparent usefulness.

In the present section of the recommendations only general principles of certain phases of the analysis will be presented. Subsequent sections will deal more in detail with special topics.

Sampling

In controlled plant production the variability of composition has not received the attention accorded to yield.

Localization of metabolic effects has been stressed by horticulturists.¹ Where such effects are confined to small tissues it becomes difficult to sample even for micro-quantitative analysis. It is generally feasible, however, to deal separately with the various organs of the plant.

Uniformity of size and appearance of individual plants and organs thereof give no certainty of uniform composition. Sufficient material must be taken to compensate variability in the plant or organ involved. The lower limits of this value must be determined experimentally and might profitably be subjected to cooperative testing. Because of the variability mentioned, as well as the requirements of certain analytical processes, it is advisable to analyze duplicate samples. When composition becomes constant with increasing size of the sample, variability has been compensated.

From twenty to one hundred plants or parts thereof, depending upon the number of uncontrolled variables in production, should suffice for a representative sample. In general, it is advisable to select at least ten times the amount of tissue to be taken as a sample for analysis. So far as possible the minimum of dry matter available from controlled plant cultures should be held at 25 grams.

¹ TUFTS, W. P. Proc. Amer. Soc. Hort. Science. 1925: 232-236.

Extraction

It should be recognized that some enzymatic changes proceed rapidly in crushed plant tissues. Expedition and relatively low temperatures² thus become important features of this treatment. The requirements probably vary with the species used and the cultural treatment.

When the extract of the fresh plant tissue is desired, this should be accomplished rapidly in a suitable mill with the addition of liberal amounts of distilled water. Plant fibers will not pass through the sieve plates of a meat chopper, but simple corrugated plates, as in the Nixtamal mill³, are effective. Qualitative tests upon the extract will determine the extent of washing necessary. The proportion of water used in the grinding process should be adjusted to the degree of succulence of the tissue. Such extracts invariably filter with difficulty, on account of colloidal constituents, but may be washed on a thick pad of paper pulp on a Buchner funnel over a vacuum flask. The filtered extract should be heated to boiling immediately to destroy enzymes, adding a slight excess of calcium carbonate to prevent hydrolysis of sugars when the extracts are appreciably acid. As colloidal material flocculates on standing, immediate analysis is preferable to preservation. Separate aliquots should be acidified with a weak acid (acetic) at the boiling point for recovery of soluble proteins.

When the solubility of colloidal materials, particularly of proteins and dextrans, may be neglected, the most generally convenient and common method of extraction is by hot alcohol. This has the advantage, of course, of immediately checking enzymatic changes of composition. Two procedures are in use. In one, sufficient 95 per cent. ethyl alcohol is used to give at least 80 per cent. concentration thereof as diluted by the water of the tissue. This volume of alcohol is heated to boiling, the cut tissue is dropped in and boiling continued for 15 minutes. Sections of the tissue should be relatively thin to favor prompt penetration of the alcohol, 2 to 3 mm. for succulent plants, and less for woody ones. If allowed to cool slowly material insoluble at room temperature will separate and may be strained off. Several washings with 80 per cent. hot alcohol treated in this manner should complete the extraction, the solutes being recovered by evaporation of the extract at low temperature. If aliquots are taken from the alcoholic extract it should be sampled at uniform temperature, as it has a considerable coefficient of expansion.

Another method includes 50 per cent. alcohol as the effective extracting agent. This has the advantage of high solvent power and in some cases has been found as effective as water. The hot extract is preserved

² NEWTON, R., BROWN, W. R., and MARTIN, W. M. *Plant Physiol.*, 1: 57-66. 1926.

³ Enterprise Mfg. Co., Philadelphia.

by increasing the alcohol concentration to about 80 per cent. Colloidal or other material which separates on cooling should be recovered with the insoluble residue. Large Buchner funnels serve conveniently for the percolation and extraction of considerable masses of tissue.

As a general value it may be accepted that somewhat less than 2 liters of water accomplishes practically complete extraction of succulent tissues. The action of hot alcohol seems to be complete at smaller volumes. It has been found that 100 grams of apple spur tissue is exhausted by about 1250 cc. of 80 per cent. alcohol. The alcoholic treatment denaturizes most proteins and renders them insoluble, but all methods of desiccation also seriously reduce the amount of soluble proteins.

The use of calcium carbonate is usually recommended in connection with extraction, chiefly to prevent the inversion of sucrose by organic acids while heating. Some workers have employed ammonia for this purpose, and it should be more effective in penetrating intact cells. This matter deserves experimental attention. In any event, it should be recognized that such additions almost invariably alter the reaction of the plant sap and are liable to disturb the natural distribution of solutes. For the latter reason also, alkalinity of the extract is to be avoided over appreciable time periods. However, an excess of solid calcium carbonate is not objectionable in this sense. To minimize alterations during the recovery of extracts from distinctly acid tissues, it is advisable to conduct a parallel extraction without the use of a neutralizing reagent.

Plant extracts should be analyzed as promptly as possible. Unavoidable delay is more permissible with alcoholic extracts than watery ones, due to the flocculation of colloids in the latter case, as well as the necessary use of preservatives. BURRELL, of Ohio State University, found values for amino and nitrate nitrogen constant in alcoholic extracts for two weeks. Exposure to light should be avoided. The insoluble residue should be oven-dried and ground as fine as feasible for further analysis, preferably to 100 mesh. For this purpose the Dreef pestle mill⁴ is unusually satisfactory.

Desiccation

Past investigation of chemical composition in plant tissues have suffered rather generally from failure to consider the effects of the method of desiccation employed. This is especially true of "air drying," where enzymatic changes may become extensive and mask the real composition of the tissues. THOMAS, of Pennsylvania State College, recommends a temperature of 60° C. in vacuo as maintaining with least disturbance the

⁴ Formerly stocked by Eimer and Amend, New York.

original composition of the tissue. SPOEHR and CHIBNALL advise that different types of plant tissue are widely variable in their requirements of desiccating treatment. LINK⁵ has discussed the factors involved in heat drying. It is not possible to avoid considerable denaturing of proteins by heat drying, and in highly acid tissues sucrose suffers inversion. In general, with masses of tissue too great for treatment in vacuo, drying at 60° C. with forced ventilation minimizes hydrolytic and respiratory changes without caramelizing the sugars. Many types of tissue permit also a preliminary heating at about 100° C. for a matter of 30 minutes to kill the tissue. Other types, rich in colloidal materials, are more resistant to desiccation and require preliminary alcoholic extraction.

Lipoid removal

The necessity of clearing cell walls from fatty and waxy components in order to facilitate extraction and the entrance of watery solvents is rather obvious and well known. To a considerable extent this function is accomplished by the hot alcoholic extraction previously mentioned. Few other lipid solvents are applicable to fresh, watery tissue and so universal in solvent effect. SANDO, of the U. S. Bureau of Plant Industry, considers alcohol the most efficient solvent for lipoids in the sense that the latter term includes fats, fatty acids, waxes, sterols and phospholipoids. Certainly this extract is of heterogeneous character and has little significance for expression in quantitative terms. It may be further fractionated by extracting the recovered solids with ether.

When dried tissue is to be dealt with, ordinary (ethyl) ether is to be preferred for extraction. The low boiling point and general handling qualities of this solvent have given it general favor. It must be anhydrous and free from alcohol, or sugars and other non-lipoidal constituents will be removed. This form is advertised by manufacturers at a reasonable increase over the cost of commercial ether. It should be possible to obtain it true to specifications. According to SANDO, the lipoidal extraction of dry material is incomplete as compared with the use of alcohol on fresh tissues. The use of ether should leave all carbohydrates and non-lipoidal nitrogenous constituents in the tissue.

Determination of moisture

Regardless of the completeness of removal of water from tissue, as usually stored and handled it acquires the "air dry" state by attaining moisture equilibrium with the contiguous atmosphere. Hence the neces-

⁵ LINK, Jour. Amer. Chem. Soc. 45: 439-447. 1923; 46: 2044-2050. 1924; 47: 470-476. 1925.

sity of determining the actual dry weight used for analysis. It is probably sufficient, as a general principle, to employ the same temperature and desiccator service throughout a single investigation or related ones. Where drying is accomplished *in vacuo* a standard value of barometric pressure should be selected for the temperature employed, as the percentage of water remaining in the tissues varies appreciably with moderate differences in pressure. In the relatively rare cases where volatile organic compounds are present in appreciable proportions their recovery in the process of drying the fresh tissue presents, of course, a special requirement.

Expression of results

The common practice of stating composition as percentages of the dry matter may fail to denote changes in the absolute amounts of constituents. In this respect the amount of constituent per plant, or multiple thereof, is advantageous. CHIBNALL⁶ stresses the possibility that variations of less abundant constituents may be masked by increases of inert wall material. In this event the basis of fresh weight will have particular value in the statement of results, unless the water content is rather constant. Perhaps the basis of dry matter plus bound water content⁷, if generally practicable of determination, will be found more significant. Physiologists are attempting to determine what bases for expressing results will best represent the concentration of metabolic materials in the living tissues under variable conditions. In view of its long-standing usage it seems advisable to retain the expression of percentages in the dry matter, employing also the basis of green weight and such others as appear particularly significant.

This report was organized by W. E. TOTTINGHAM for the Committee.

C. O. APPLEMAN,

W. E. LOOMIS,

T. G. PHILLIPS,

W. E. TOTTINGHAM (chairman),

J. J. WILLAMAN.

⁶ Ann. Bot. 37: 511-518. 1923.

⁷ NEWTON, R., and GORTNER, R. A. Bot. Gaz. 74: 442-446. 1922.

THE USE OF POTASSIUM OXALATE AS A DELEADING REAGENT*

W. E. LOOMIS

Two deleading reagents are recommended in the Association of Official Agricultural Chemists' Methods of Analysis (1), anhydrous sodium carbonate, and potassium oxalate. In addition, sodium sulphate, sodium phosphate, and hydrogen sulphide are advocated by various writers for a number of conditions.

SAWYER (8) objected to the use of sodium carbonate for deleading because an excess of the reagent gives an alkaline reaction in which precipitated lead salts are redissolved and levulose is destroyed, even at low concentrations. The addition of the carbonate in such a manner as to avoid an excess is a difficult and tedious procedure. Sodium sulphate is more stable but lead sulphate is precipitated slowly in very fine crystals which are filtered out with some difficulty. Additional crystals frequently form in the filtrate if it is allowed to stand. Sodium phosphate and hydrogen sulphide are effective in removing soluble lead but leave the solution acid and are consequently not well adapted for use where reducing and non-reducing sugars are to be determined separately. Potassium oxalate has been advocated by a number of writers. Of these SAWYER stressed the neutrality of the cleared solution and the convenience with which this reagent is used. MEAD and HARRIS (6) and EYNON and LANE (4) considered the use of potassium oxalate desirable because of its action in precipitating the alkaline earth metals. They found that this group of substances lowered the reducing power of sugar solutions to which they were added.

Experimental results

EFFECT OF THE DELEADING REAGENT UPON THE P_H OF THE CLEARED SOLUTION.—When quantities of 1.25 sp. gr. neutral-lead-acetate solution varying from 1 to 5 cc. were removed from sugar or plant extract solutions by the addition of an excess of potassium oxalate, the filtrate was found in every case to give a reaction color value falling between 6.8 and 7.2 P_H when tested with LA MOTTE standard indicators and compared with the CLARK and LUBS color chart (3). When hydrogen sulphide was used as the deleading reagent the acidity of the filtrate varied with the quantity of lead

* The work reported here was done at Cornell University during the tenure of a National Research Council Fellowship in the Biological Sciences.

acetate removed and for 5 cc. of solution gave P_H values as low as 4.8. It should be stated that there was no apparent inversion of the non-reducing sugars in the extracts of green celery leaves when they were delead with hydrogen sulphide and boiled for ten minutes to expel the excess of the gas. Celery leaves collected during the day are high in sugars and about 80 per cent. of these are non-reducing. The P_H of the cooled filtrate was found to be 5.2. With sodium carbonate a distinctly alkaline reaction was obtained by adding an excess of salt above that required to precipitate the lead acetate present. If these alkaline solutions were heated or allowed to stand at room temperature there was a change in color, sometimes the development of a distinct odor, and a rapid destruction of reducing sugars.

EFFECT OF AN EXCESS OF POTASSIUM OXALATE UPON THE REDUCTION OF CUPRIC COPPER.—Bureau of Standards dextrose and "Difco" levulose were used in these tests. One gram of Baker's potassium oxalate crystals was added to 50 cc. of sugar solution and reduction carried out under QUISUMBING and THOMAS conditions (7). The cuprous oxide was first weighed directly and its purity was then determined electrolytically by the official sulphuric-nitric acid method. Check clearings in which 1 cc. of neutral lead acetate solution was removed by a moderate excess of oxalate, are included for comparison. All data are an average of triplicate determinations which varied by less than one per cent.

TABLE I

THE EFFECT OF POTASSIUM OXALATE ON THE REDUCTION OF COPPER BY REDUCING SUGAR SOLUTIONS

REDUCING SOLUTION	Cu_2O PRECIPITATED	SUGAR INDICATED	METALLIC COPPER	SUGAR INDICATED	COPPER IN Cu_2O
	mg.	mg.	mg.	mg.	Per cent.
Levulose solution	399.9	183.0	352.5	181.2	88.15
Levulose plus 1 gm. potassium oxalate	400.5	183.2	352.9	181.4	88.11
Levulose plus lead and potassium oxalate	401.3	183.6	353.6	181.7	88.11
Dextrose solution ...	293.9	131.7	260.1	131.1	88.53
Dextrose plus 1 gm. potassium oxalate..	293.7	131.6	260.3	131.2	88.62
Dextrose plus lead and potassium oxalate	294.2	131.8	260.7	131.4	88.61

No indication is given by the data in table I of any effect on copper reduction from the small excess of potassium oxalate required in deleading, or from an excess approximately eight times as great.

THE CO-PRECIPITATION OF REDUCING SUGARS BY LEAD OXALATE.—Oxalates were included by BRYAN (2) among the salts causing the precipitation of reducing sugars in the presence of basic lead acetate. In a previous paper (5) it has been shown that reducing sugars are co-precipitated in the presence of basic lead acetate with any amorphous precipitate, but that they are not precipitated to an appreciable extent in the absence of the lead-oxide radicle. This is illustrated in table II, where the reducing-sugar content of various sugar solutions and plant extracts treated with 1 and 5 cc. of 1.25 sp. gr. neutral lead acetate is compared after removing the excess of lead with potassium oxalate. Because of slower crystallization in the heavier precipitate, the volume of the lead oxalate from the 5 cc. treatment was about ten times that of the 1 cc. The last column of table II shows that there was no significant loss from either the excess of neutral lead acetate used or the heavy lead-oxalate precipitate.

TABLE II

LEAD OXALATE AS A CO-PRECIPITANT OF REDUCING SUGARS

SAMPLE	REDUCING SUGARS		RECOVERY WITH EXCESS TREATMENT
	1 CC. NEUTRAL LEAD ACETATE + POTASSIUM OXALATE IN EXCESS	5 CC. LEAD ACETATE + POTASSIUM OXALATE IN EXCESS	
	mg.	mg.	Per cent.
Mangel extract no. 3	43.2	43.9	101.63
Mangel extract no. 4	72.9	72.4	99.31
Mangel extract no. 2	28.9	28.8	99.65
Mangel extract no. 6	41.3	41.6	100.76
Sweet potato extract	56.3	55.5	98.58
Celery leaf extract	154.2	154.2	100.00
Celery stalk extract	29.0	29.8	102.76
Tomato leaf extract no. 1	40.2	40.1	99.75
Tomato leaf extract no. 2	26.1	26.0	99.62
Applewood extract no. 3	93.2	91.7	98.39
Applewood extract no. 2	158.9	157.6	99.18
Pure fructose solution	183.0	182.4	99.67
Pure dextrose solution	132.1	130.8	99.01
Average	81.48	81.14	99.87

POTASSIUM OXALATE AS A LEAD PRECIPITANT.—If hydrogen sulphide is passed through a cleared solution delead with potassium oxalate, a black precipitate of lead sulphide is formed, indicating the incomplete removal of lead. Quantitative tests showed that of 184.8 mgs. of lead added to a mangel extract in 1 cc. of neutral lead acetate, 81.8 mgs. were carried down with the clearing precipitate and 100.8 mgs. were removed from the filtrate by the addition of an excess of potassium oxalate. Obtaining the lead remaining in the delead solution by difference, we find that 2.2 mgs. of lead or 2.14 per cent. were not removed. This incomplete removal of lead probably accounts for the loss in reduction when a solution delead with potassium oxalate is heated before making the reduction determinations. The data on the clearing of pure sugar solutions given in table I, show that the small quantity of lead left by potassium oxalate does not affect the reducing power of the solution under normal conditions. The same conclusion must be drawn from table III, where the reducing powers of solutions delead with potassium oxalate and hydrogen sulphide are compared.

TABLE III

A COMPARISON OF POTASSIUM OXALATE AND HYDROGEN SULPHIDE AS DELEADING REAGENTS

SOLUTION OR EXTRACT	LEAD ACETATE USED	DEXTROSE	
		DELEADED WITH POTASSIUM OXALATE	DELEADED WITH HYDROGEN SULPHIDE
		mg.	mg.
Applewood extract	2 cc. neutral	43.2	43.2
Tomato extract	1 cc. neutral	26.1	25.9
Mangel extract	1 cc. neutral	28.9	28.6
Mangel extract	5 cc. basic	33.9	33.5
Sweet potato extract	5 cc. basic	54.2	53.0
Dextrose solution	5 cc. neutral	130.8	130.6

Summary and recommendations

Potassium oxalate is a convenient deleading reagent for use in clearing plant extracts. It leaves the cleared solution neutral to litmus and an excess considerably greater than that required to give rapid flocculation of the lead-oxalate precipitate, has no effect on the reducing power of the solution.

Potassium oxalate does not precipitate all of the lead present and solutions delead with this reagent cannot be heated without lowering their

reducing power. Under normal conditions, however, solutions delead with potassium oxalate give very slightly higher reducing values than do those delead with hydrogen sulphide, which removes practically all of the lead, because the lead-oxalate precipitate is more easily washed than is the precipitate of lead sulphide.

It is recommended that a quantity of potassium-oxalate crystals, which is estimated to be 0.2 to 0.5 gms. in excess of the quantity necessary to remove the lead from the solution being cleared, be placed in a beaker and the cleared solution be filtered onto the crystals. This method insures rapid deleading and the high initial concentration of oxalate coupled with the time required to filter and wash the clearing precipitate allows for a partial crystallization of the lead oxalate so that it may be rapidly filtered and washed.

UNIVERSITY OF ARKANSAS,
FAYETTEVILLE, ARKANSAS

LITERATURE CITED

1. Association of Official Agricultural Chemists. *Methods of Analysis*, revised. Washington. 1924.
2. BRYAN, A. H. Report in U. S. D. A. Bureau Chem. Bull. **116**: 73. 1907.
3. CLARK, W. M. The determination of hydrogen ions. Williams and Wilkins. Baltimore. 1920.
4. EYNON, L. and LANE, J. H. The influence of alkaline earths on the determination of reducing sugars by Fehling's solution. *Jour. Soc. Chem. Ind.* **42**: 143T-146T. 1923.
5. LOOMIS, W. E. A study of the clearing of alcoholic plant extracts. *Plant Physiol.* **1**: 179-189. 1926.
6. MEAD, G. P. and HARRIS, J. B. The gravimetric determination of reducing sugars in cane products. *Jour. Ind. Eng. Chem.* **8**: 504-509. 1916.
7. QUISUMBING, F. A. and THOMAS, A. W. Conditions affecting the quantitative determination of reducing sugars by Fehling's solution. *Jour. Amer. Chem. Soc.* **43**: 1503-1526. 1921.
8. SAWYER, H. E. Potassium oxalate as a lead precipitant in sugar analysis. *Jour. Amer. Chem. Soc.* **26**: 1631-1635. 1904.

AQUEOUS EXTRACTS OF SEEDS AS AGENTS IN THE PREPARATION OF SILVER SOLS

E. V. MILLER AND R. P. HIBBARD

Introduction

Many methods have been suggested for the preparation of colloidal metals by condensation with the use of organic compounds as stabilizers (1). Among these compounds may be mentioned gum arabic, gelatin, sugar, glycerol, sodium citrate, saponin, barium arabinat, sodium protalbinat, and sodium lysalbinat.

Probably colloidal silver has received as much attention as has colloidal gold. Wool-fat has been employed as a stabilizing agent in preparing colloidal silver from organic solutions. KOHLSCHÜTTER (2) reduced AgOH by means of hydrogen. LÜPPO-CRAMER (4) obtained a series of beautifully colored silver sols by the reduction of AgNO_3 with hydrochinone in the presence of gelatin. CAREY LEA (3) reduced AgNO_3 with ferrous citrate, dissolving the deposit in water and reprecipitating with ammonium nitrate.

SVEDBERG (6) observed that a silver plate submerged in water or alcohol produced a silver colloid when illuminated by ultra-violet light or by X-rays. According to TRAUBE-MENGARINI (7) a certain amount of silver colloid may be produced by boiling silver in water.

NORDENSEN (5) showed that silver is oxidized by both water and alcohol and is dissolved as AgOH or some other compound. This silver solution, SVEDBERG has shown, may be reduced by illumination, while TRAUBE-MENGARINI produced similar results with traces of reducing agents. The dissolution (or oxidation) is accelerated by light, especially ultra-violet light.

The method for preparing colloidal silver described in this paper depends upon the ability of aqueous extracts of seeds to reduce AgNO_3 solutions. A number of methods for preparing colloidal silver have already been mentioned. This is an addition to the list and is highly recommended because of its simplicity of manipulation. It should therefore be of more than passing interest to plant physiologists, botanists, and chemists.

Methods

One gram of timothy seeds is stirred into 100 cubic centimeters of distilled water and allowed to stand about an hour. The solution is filtered and two drops $\text{N}/10$ AgNO_3 added. If the solution is then permitted to stand in diffused light for about half an hour a dark brown color will appear, this being due to colloidal silver. The speed of this reaction may

be increased by exposure to sunlight. However, too great exposure will precipitate the silver. If the aqueous extract is placed over a Bunsen burner and heated as soon as the AgNO_3 has been added, the colloidal silver will form within a few minutes.

If the colloidal silver solution is not placed in the strong sunlight the sol remains indefinitely stable. A solution in the laboratory at present has held up for over nine months.

Seeds investigated

The following seeds were later employed for reducing the silver, positive results being obtained in all cases except clover: Pea, bean, tomato, corn, wheat, buckwheat, grass, clover, sunflower, lettuce and beet. Later investigation revealed that though there was a precipitate formed in the clover solution, the supernatant liquid was colored a faint brown like that of other sols.

In a subsequent experiment colloidal silver solutions were prepared from the following: Corn, oats, wheat, rice, peas, beans, soy beans, cotton, beet, and gladiolus (bulb). In this case 50 and 100 seeds were soaked in 100 cubic centimeters of water. These were all placed under the ultra-microscope and were found to exhibit Brownian movement. In the sol prepared from the oats the particles were so numerous that the field presented the appearance of a confused mass of seething particles.

The particles of course are electrically charged, as was demonstrated by use of two platinum electrodes, placed on each side of the ultra-microscopic field and connected to storage batteries by means of a key. If the circuit were closed while the field was being observed the charge of the particles could be determined by their behavior in the electric field. All of the sols described possessed negative charges.

An outstanding feature of these colloidal solutions is the elaborate coloring. Colors varying from a dark amber to a rich rose or orchid were obtained. It is possible that different classes might be characterized by the color of their colloidal solutions. Corn, wheat, oats and rice all produced colloidal silver in a rose-colored suspension. Peas, beans, and soy beans were all characterized by a brown color. Other seeds were examined but could not be compared because no other closely related groups were included. These differences in color, especially the roseate hues, may depend in part on adsorption by the colloidal silver particles, as the usual color of silver sols is.

In an effort to locate the exact source of this reducing substance different parts of bean seeds were soaked in water and the solutions treated with AgNO_3 . Beans were soaked in distilled water over night. The next morning they were taken from the water, the seed coats removed, and the plumule

and hypocotyl cut out of the cotyledons. These three parts (seed coat, cotyledons, and hypocotyl and plumule) were again soaked over night. On the following morning AgNO_3 was added to the extracts.

The intensity of the colloidal silver color was greatest with the seed coats, next with the cotyledons, and least with the hypocotyl and plumule. In the last the color did not form at all until after several hours.

Nature of the reducing substance

About ten cubic centimeters of the aqueous-extract of timothy seed were enclosed in a collodion sack and lowered into a beaker of distilled water. Two or three drops of $\text{N}/10 \text{ AgNO}_3$ were added to this distilled water and in a few days the extract within the collodion sack had assumed a brown color characteristic of the colloidal silver. This would indicate non-diffusibility on the part of the reducing substance.

A more concentrated solution of the timothy extract presents a dark brown color, indicative of tannin. In the presence of ferric chloride a faint blue-black precipitate is formed, the intensity of the color resembling that produced by a .001 per cent. tannin when treated in the same way.

Following this a timothy extract was treated with lead acetate to remove the proteins. The excess lead was removed with H_2S . A few drops of AgNO_3 were added and a brown colloid, similar to the original, was formed. However, the colloid soon settled out.

The experiment was repeated, first adding enough tannin to make it a .001 per cent. solution. The same brown colloid was formed, appearing permanent at first, but settling out after a longer interval. The filtrate did not reduce Fehling's solution.

The instability of this last colloid was no doubt largely due to the presence of acid formed by the method employed for removing the excess lead. Later sodium carbonate was used instead of the hydrogen sulphide and the filtrate treated with AgNO_3 . This time a colloidal silver formed which was not quite the same color as the original. This colloid lasted longer than the others (about a week) but finally settled out like the rest.

Difficulty in identifying this reducing substance arises from the fact that many substances are extracted from the seeds by both water and alcohol. In some instances sugar has been found present and in others it has not.

An alcoholic (90 per cent.) extract of corn meal has been found to reduce the silver nitrate, producing a colloid identical in appearance with the original one. In previous cases the reduction has been brought about by an aqueous extract. Furthermore, this alcoholic extract has produced a silver sol which thus far rivals the aqueous extract in stability.

In another experiment an alcoholic extract of corn meal was evaporated and the alcohol replaced with water as the alcohol evaporated. The zein was precipitated in this process. This impure zein was dissolved in ethyl alcohol and AgNO_3 added to the solution. A silver sol, very similar to the original, was formed. After a period of a week this sol became cloudy and appeared to be precipitating.

From the above results it seems possible that both zein and sugar would be extracted by the alcohol. When the alcohol is replaced by water the sugar would go into solution, leaving the zein to be precipitated. Hence it might be surmised that the silver can be reduced by either the alcohol- or water-soluble proteins and the sol stabilized by sugar or *vice versa*.

An aqueous extract of corn meal was made after the meal had first been extracted with 90 per cent. alcohol. This aqueous extract reduced Fehling's solution when tested for sugars. There were also proteins present which were removed before testing for sugars. A colloidal silver solution was produced by this aqueous extract but the sol was unstable.

The zein mentioned in this paper has been designated as "impure zein." It was prepared by extracting corn meal with alcohol, evaporating off the alcohol, replacing the alcohol with water as the alcohol evaporated, and collecting the zein as it precipitated.

A little later some pure zein was prepared. An alcoholic extract of corn meal was made, the zein salted out with dilute NaCl , and the NaCl removed by dialysis. It is possible that some of the carotinoid pigments were carried down with the zein but we shall distinguish between the two by calling this last one "pure zein."

A stable colloid has been prepared by dissolving this "pure zein" in alcohol and adding the AgNO_3 . With the impure zein the colloid was not stable unless a small amount of arabinose was added.

Summary

A simple method has been described for preparing colloidal silver from aqueous extracts of seeds. The process is essentially one of reduction or condensation.

Further investigation revealed three other methods for preparing colloidal silver. They are as follows: (1) Alcoholic extract of seeds; (2) Alcoholic solution of pure zein; (3) Alcoholic solution of impure zein plus arabinose.

The reducing substances are not as yet known but it seems probable that they might be alcohol- and water-soluble proteins with sugar as the stabilizer.

The work on this problem has been made possible by the encouragement and financial aid of the D. M. Ferry Seed Company of Detroit.

MICHIGAN STATE COLLEGE,
EAST LANSING, MICHIGAN

LITERATURE CITED

1. BANCROFT, W. D. Applied Colloid Chemistry. p. 172. 1921.
2. KOHLSCHÜTTER, B. On the reduction of silver oxide by hydrogen and on colloidal silver. *Zeitschr. Elektrochem.* **14**: 49-63. 1908.
3. LEA, M. CAREY. On allotropic forms of silver. *Amer. Jour. Sci.* (3) **37**: 476-491. 1889.
4. LÜPPO-CRAMER, H. Colloidal chemistry and photography. *Zeitschr. Chem. Ind. Kolloide* **7**: 99-103. 1910.
5. NORDENSEN, H. The spontaneous formation of colloids of metals in contact with a solvent. *Kolloidchem. Beih.* **9**: 91-109. 1915.
6. SVEDBERG, THE. The formation of dispersed systems through the action of ultra-violet and Röntgen rays on metals. *Zeitschr. Chem. Ind. Kolloide* **6**: 129-136. 1910.
7. TRAUBE-MENGARINI, M., and SCALA, A. The formation of colloidal solutions of the noble metals in boiling distilled water. *Zeitschr. Chem. Ind. Kolloide* **6**: 65-90, 240-250. 1910.

BRIEF PAPERS

CLIMATIC EFFECTS IN THE METABOLISM OF MAIZE*

These brief notes relate to preliminary results with maize by methods similar to those used in a previous study of the sugar beet.¹ Particular interest attaches to the investigation of this plant because of its comparative independence from climatic effects in composition of the grain, as previously shown.²

In the present test composite samples of leaf, stalk and ear tissue were taken from fifty plants six times in a period of ten days during mid-August. These were preserved in hot alcohol, with the addition of calcium carbonate. The extract was recovered in the usual manner and both extract and residue were subjected to chemical analysis by conventional methods.³ In view of its limited scale, detailed presentation and discussion of the work would be untimely, but certain results seem to be of considerable significance.

The percentage of sucrose in the dry matter of the leaf greatly exceeded that of reducing sugars. Both sucrose and dextrins varied in general with the solar radiation. Sugars in the stalk and ear varied independently of their proportions in the leaf, and the same was true of mono-amino acids. On the other hand, the composition of the stalk was relatively stable and correlated with that of the ear.

The accompanying table gives a comparison of composition in leaves of maize with that of other species grown adjacent in the field with similar cultural treatment and sampled simultaneously. Varieties used were: Yellow Dent maize, Marquis wheat and Black Amber sorghum.

Despite its relative maturity (early heading stage) the wheat contained much higher proportions of total soluble nitrogen and of protein nitrogen than did maize. On the other hand, sorghum had much the same composition as maize. This is in harmony with its relative stability of composition with varying climate.

Of the two types of composition in tissue adapted largely to synthetic processes one would expect that which included the more stable compounds

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station, Madison.

¹ TOTTINGHAM, W. E., LEPKOVSKY, S., SCHULZ, E. R., and LINK, K. P. Climatic effects in the metabolism of the sugar beet. *Jour. Agric. Res.* 33: 59-76. 1926.

² TOTTINGHAM, W. E. Physiological stability in maize. *Science (New Series)* 59: 69-70. 1924.

³ Official and tentative methods of analysis, Association of Official Agricultural Chemists. Washington, D. C., 1919. pp. 5-7, 78-79, 81, 95-96.

TABLE I
COMPARATIVE COMPOSITION OF LEAVES

PLANT SPECIES	DATE OF SAMPLING	DRY MATTER	TOTAL NITROGEN IN DRY MATTER	PARTIAL DISTRIBUTION IN THE TOTAL NITROGEN	
				TOTAL SOLUBLE N	SOLUBLE PROTEIN
		Per cent.	Per cent.	Per cent.	Per cent.
Maize	7/15/24	25.7	3.8	18	5
Wheat	7/15/24	41.5	3.4	35	15
Maize	7/17/24	23.1	3.7	27	14
Wheat	7/17/24	34.8	3.1	55	32
Maize	7/16/25	27.2	3.5	26	14
Sorghum	7/16/25	24.4	4.0	30	17
Maize	7/22/25	27.2	3.5	37	26
Sorghum	7/22/25	26.0	4.1	41	27

to be the more resistant to disturbance by climatic variations. Maize and sorghum leaves possess the common factor of low proportions of total soluble nitrogen and soluble protein, as contrasted with high values of these factors in wheat and the sugar beet. Furthermore, maize is characterized by low proportions of reducing sugars, while the sugar beet leaf contained much more reducing sugar than sucrose in our tests. MILLER'S⁴ results show the same status of sugars in sorghum as in maize, while such data as we have from greenhouse cultures place wheat in the category of the sugar beet.

There are not sufficient grounds for imputing to this difference in composition the chief cause of stability of corn and sorghum against climatic influences. On the other hand, it may be ultimately important to consider that sucrose and less soluble forms of protein are quite likely to be less altered by the effects of temperature and other climatic factors than are such reactive compounds as glucose, soluble proteins and other soluble nitrogenous compounds.—W. E. TOTTINGHAM and H. W. KERR, *The University of Wisconsin*.

⁴ MILLER, E. C. Daily variation of the carbohydrates in the leaves of corn and the sorghums. Jour. Agric. Res. 27: 785-808. 1924.

A DEVICE FOR MAINTAINING CONSTANT LEVEL OF CULTURE SOLUTIONS

(WITH ONE FIGURE)

In growing certain woody plants in continuously flowing culture solutions it has been found essential to submerge only a portion of the established root system while still maintaining a relatively high humidity around the unsubmerged roots. The following apparatus has been found especially satisfactory for this purpose, allowing the use of ordinary jars of glass or other material as culture vessels without alteration. The arrangement in use is shown in the accompanying figure.

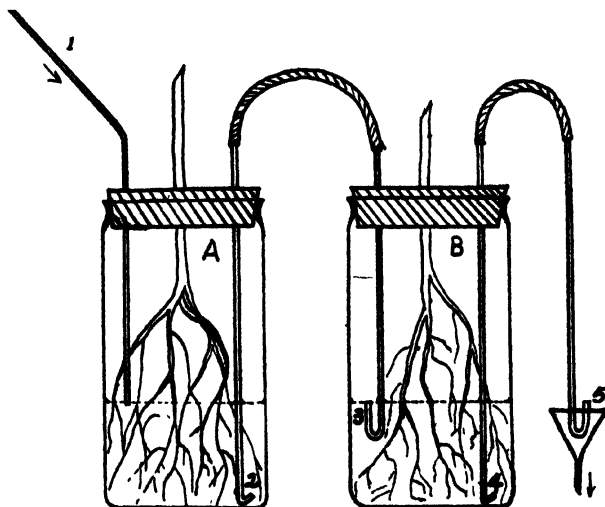


FIG. 1. Constant level device for culture solutions. Description in text.

The tube 1 leads from a supply reservoir into jar A, ending near the surface of the solution. The tube 2-3 is a siphon having a j-bend at 3 with the shorter arm 2 or 3 inches long. Tube 4-5 is a similar siphon leading from jar B and emptying into a receiving jar. The ends of the siphons at 2 and 4 may be at any level below the openings at 3 and 5, but are shown extending to the bottoms of the jars, an arrangement which aids in the circulation within the jars, since the inflow occurs at the surfaces of the solutions. The short bends in the tubes at 2 and 4 are to prevent entrance of gas bubbles which would break the siphon streams.

When the jars are filled to the levels shown and the siphons are filled and solution is allowed to enter A at the desired rate through tube 1, it automatically flows through the siphon into B, whence it is likewise removed through the second siphon and passes into the receiver. If the solution stops entering from tube 1, the whole system stops but the siphons do not break on account of the j-bends at 3 and 5; flow starts again automatically when solution flows into A. The level of solution in A and B is determined by the height of the openings at 3 and 5. The level of solution in B may be maintained the same or lower than that in A, but not higher. As many jars as desired may be operated in series.—F. G. ANDERSEN, *Pomology Laboratory, University of California.*

NOTES

International Congress of Plant Sciences.—The Ithaca Congress has passed into history, and is generally conceded to have been the most valuable meeting of botanists ever held in America. The meetings had been planned with the utmost care, and every feature of the great gathering evidenced the thoughtfulness and thoroughness with which the preparations had been made. The attendance far exceeded expectations and fell but little short of 1,000. The attendance from foreign lands made only a fraction of the total, but was very satisfactory. Everyone regretted the absence of the French botanists from the meeting but nearly all the important nations were represented by delegates. It was particularly gratifying to see the large Russian delegation at the Congress.

Although no legislation was attempted at this Congress, it has paved the way for other international gatherings, the next of which will be held in London in 1930.

The physiological meetings of the Ithaca Congress were very well attended, and the papers presented were generally of high merit. It is a pleasure to be able to present a fairly full account of the physiological proceedings to our readers in this number of *PLANT PHYSIOLOGY*.

Society Dues.—The constitution of the American Society of Plant Physiologists provides for a fiscal and official year beginning July 1, and ending June 30. The journal year, however, begins January 1. The dues paid during the last half of 1925 entitle the member to all of the issues of *PLANT PHYSIOLOGY* for 1926, and the dues now payable are for the 1927 issues of the journal, and for membership from July 1, 1926, to June 30, 1927. By this arrangement the secretary-treasurer has six months in which to prepare the mailing list for the following calendar year. New members joining the Society at this time should remit \$10.00 if they desire to secure the first volume of *PLANT PHYSIOLOGY*. All physiologists are urged to secure the first volume now, as it will soon be unobtainable in complete sets.

Protoplasma.—An international journal of the physical chemistry of protoplasm is announced by Gebrüder Borntraeger, of Berlin. The ground to be covered includes the colloid chemistry of protoplasm; physico-chemical properties of protoplasts, such as surface tension, viscosity, plasticity, swelling, elasticity, adhesion, adsorption and acidity; microchemistry and electrometry of protoplasts; structure of living protoplasm; osmotic phenomena of protoplasts; permeability, plasmolysis, narcosis, cytolysis, hemolysis, vital staining; physico-chemical causes of protoplasmic movement; microdissec-

tion; ultramicroscopic and polarization studies of protoplasm; mechanism of cell and nuclear division; protoplasmic activation; physico-chemical problems of pharmacological and toxic effects; resistance and sensitiveness to radiations; physical-chemistry of pathological cells, as of tumors; and studies of non-living colloidal models which throw light on protoplasmic behavior.

The editors are JOSEF SPEK, of the Zoological Institute, Heidelberg University, and FRIEDL WEBER, of the Plant Physiological Institute, University of Gratz. At present manuscripts should be sent to Dr. F. WEBER only.

We extend good wishes to the new journal. Plant physiologists will no doubt find much valuable material appearing in *Protoplasma*, and need it in both private and public libraries.

Author Abstracts.—The editor of Biological Abstracts has requested the cooperation of PLANT PHYSIOLOGY in securing abstracts for the papers appearing in this journal. The simplest and quickest way to have the abstracts appear is to have author abstracts accompany manuscripts when they are first submitted for consideration by the editors of PLANT PHYSIOLOGY. In order to assist the editors of Biological Abstracts in the enormous task of securing abstracts of all botanical literature, we are therefore requesting all authors of papers to furnish with the manuscript submitted a brief, accurate abstract which may be forwarded to Biological Abstracts at the time of publication. This will insure the earliest possible appearance of the abstracts, and should provide better, more intelligent abstracts than any other method. The main difficulty is that authors sometimes think their material is much more valuable than it is, and make abstracts too long and detailed. The abstracts should be very brief, and right to the point. Most papers can be adequately abstracted in 100 to 200 words. We expect all of our contributors to comply with this request.

The University of Minnesota Section.—Permanent organization of the University of Minnesota Section of the American Society of Plant Physiologists is reported as follows: Local vice president, Professor A. C. ARNY, University Farm, St. Paul, Minnesota; second vice president, Professor L. O. REGEIMBAL; secretary-treasurer, Professor A. H. LARSON. In accordance with the constitutional provision, Professor ARNY becomes *ex-officio* a member of the Executive Committee of the Society. The new section has the cordial good wishes of all who are interested in strengthening the bonds of good fellowship, and encouraging the resourcefulness of plant physiologists in carrying forward their research.

Photosynthesis.—It is a pleasure to record the publication of a book by this title from one of the best known students of the chemistry of the process, Dr. H. A. SPOEHR, of the Carnegie Institution. The book takes up particularly those phases of the subject which look toward the explanation of the plant's unique power to act as a converter of radiant sunlight energy into potential chemical energy of food and fuel. Inasmuch as it is difficult to draw many conclusions in the present state of our knowledge, the author gives more attention to the results of experimental investigations of the chemistry of the process than to the conclusions which have been derived from field observations and empirical studies. The book is suggestive, as the author gives special attention to features which offer promise of results along experimental lines. All physiologists will want this excellent book. It is published by the Chemical Catalog Co., New York. Price, \$6.50.

Colloid and Capillary Chemistry.—A particularly valuable service has just been rendered by H. S. HATFIELD in translating the third German edition of FREUNDLICH'S Colloid and Capillary Chemistry into the English language. This will make more available one of the truly great works in this field. It is an excellent translation of FREUNDLICH'S masterpiece, and needs no description. The book, 883 pp., price \$14.00, is published by E. P. Dutton and Co., New York.

Surface Equilibria.—Attention is directed to the publication of a book on Surface Equilibria of Colloids by P. LECOMTE DU NOÛY. This book will be of considerable interest to physiologists generally, from the interesting and valuable technique which DU NOÛY has developed, and from the rather far reaching conclusions which he draws with reference to the colloidal systems of organisms as the result of his measurements of surface equilibria. The Chemical Catalog Co., New York, is the publisher. Price, \$4.50.

Physikalische Chemie der Zelle und der Gewebe.—The sixth edition of this indispensable work has appeared from the Engelmann Press, Leipzig, 1926. Nearly all the chapters have been modified somewhat by the rapid advances made since the fifth edition was published two years ago.

Physical Chemistry and Colloid Chemistry.—A condensed introduction to the physical and colloid chemistry most needed by students of plant and animal physiology and medicine, with the mathematics made unusually clear, has been prepared in German by the recent translation of KRUYT'S little book from the second Dutch edition. It is translated by NOWAK, and published by the Akademische Verlagsgesellschaft, Leipzig, 1926. This little Einführung in die physikalische Chemie und Kolloidchemie will prove

very helpful in getting hold of the fundamentals. Incidentally it furnishes good practice in reading German. It is clearly written, and not too difficult.

Plant Nutrition and Plant Production.—Under this title the University of California Press has published the Hitchcock Lectures of 1924, which were delivered by Sir JOHN RUSSELL, Director of the Rothamsted Station in England. There are five chapters to this excellent book, as follows: The study of plant nutrients; positive science and exact demonstration; decay and the living plant, *mors janua vitae*; the soil microorganisms, can they be controlled and utilized; and the soil and the living plant. The historical introduction follows the lines of Soil Conditions and Plant Growth. As is true of all of Sir JOHN RUSSELL's writings, the story of plant nutrition is fascinatingly told. Plant physiologists will all want to read this delightful summary, written by one of the world's eminent scientists. The book is beautifully illustrated, with 37 figures and 21 full-page plates. The plates alone are worth the price of the book. There is a vast deal of sound philosophy incorporated into the story, for Sir JOHN RUSSELL is more than an investigator; he is a student of life in its broadest aspects, and a philosopher of unusual keenness of insight. This book should have a wide circulation. The price is \$2.50, and can be obtained from the University of California Press, Berkeley, California.

GENERAL INDEX

A

Abstracts, author, 420
 Accumulation of dyes, 215
 Acids, fatty, oxidation of, 349
 Adsorption and relative hardness, 165
 American Association for the Advancement of Science, 209
 Analysis, chemical, of plant tissues, 397
 Analytical methods, committee, 291
 ANDERSEN, F. G., Constant level for culture solutions, 417
 ANDREWS, F. M., Food storage, 287
 Apple, Grimes, ripening and storage changes, 251
 relative hardness and adsorption, 165
 APPLEMAN, C. O., Analysis of plant tissues, 397
 Author abstracts, 420
 Auto-irrigator, adjustment and wheat growth, 387
 Auximones, 273

B

BAKKE, A. L., Adsorption and hardness in apples, 165
 BARNES, CHARLES REID, life memberships, 206
 Bibliographies, chemical, 211
 Boron, indispensable for higher plants, 231
 BOSE, J. C., Nervous mechanism of plants, 291
 BROWN, W. R., Plant tissue fluids, 57

C

Cane, sugar, distribution of roots, 363
 Cell, conductivity, for continuous respiration, 205
 Cells, living, accumulation of dyes, 215
 CHANDLER, W. H., Fruit growing, 93
 Chemical action of ultraviolet rays, 212
 analysis of plant tissues, 397
 bibliographies, 211
 Citation of literature, 206
 CLARK, NORMAN ASHWELL, Reproduction of *Lemna*, 273

Clearing, plant extracts, 179
 Climatic effects, in metabolism of maize, 415
 Clovers, resistance to low temperatures, 281
 COLES, HAROLD W., Digestion of pectin and methylated glucoses, 379
 Colloid chemistry, FREUNDLICH, 421
 and physical chemistry, KRUYT, 421
 Colorimetric methods, for nitrates, phosphates, potassium, 191
 Committee, analytical methods, 291
 program, 290
 Conductivity, cell for continuous respiration, 205
 Congress, international, of plant sciences, 93, 209, 289, 293, 419
 Ithaca, plant physiology at, 293
 Constant level, for culture solutions, 417
 Correlation, between vegetative and reproductive functions, 3
 localization of effects, 36
 Critical tables, international, 291

D

Deleading, potassium oxalate, as agent, 403
 Digestion, of pectin and methylated glucoses, 379
 Drought resistance, physiological basis, N. A. MAXIMOW, 213
 Dues, society, 419
 DUNN, STUART, Adsorption and hardness, 165
 DU NOÛY, P. LECOMTE, Surface equilibria, 421
 Dyes, accumulation of, in living cells, 215

E

EATON, SCOTT V., and EATON, J. H., Sulphur in rainwater, 77
 ELLIS, C., Chemical action of ultraviolet rays, 212
 Endowment fund, 290
 Equilibria, surface, DU NOÛY, 421
 Extraction of plant tissue fluids, 57
 Extracts, seed, for silver sols, 409

F

- Fatty acids, oxidation, 349
 FLEU, CONYERS B., JR., Seeds, 94
 Food, storage, 287
 Foreword, 1
 FREUNDLICH, H., Colloid and capillary chemistry, HATFIELD translation, 421
 Fruit growing, W. H. CHANDLER, 93
 Fruits, growth studies, 265
 FULMER, ELLIS I., Growth of yeast, 67
Fusarium lini, nutritional studies, 151

G

- GERHARDT, FISK, Ripening and storage changes in Grimes apple, 251
 Germination, oxygen requisite for, 95
 GILBERT, B. E., Colorimetric methods for nitrates, phosphates, potassium, 191
 Glucoses, methylated, digestion of, 379
 Grimes apple, ripening and storage changes, 251
 Growth of wheat in auto-irrigated soils, 387
 of yeast, 67
 promoting substances and *Lemna*, 273
 studies on fruits, 265
 GUSTAFSON, FELIX G., Growth studies, 265

H

- Hardiness, relative, and adsorption, 165
 HARVEY, R. B., Conductivity cell for continuous respiration, 205
 HATFIELD, H. S., Translation of FREUNDLICH, colloid and capillary chemistry, 421
 Hawaiian Islands, sugar cane root distribution in soils of, 363
 HEMMI, TAKEWO, Growth of wheat in auto-irrigated soils, 387
 HIBBARD, R. P., Silver sols, 409
 HUTCHINS, LEE M., Oxygen-supplying power of soils, 95
 Hydrogen ion concentration and reproduction of *Lemna*, 273
 Hydrolysis, of starch by polarized light, 201

I

- International Congress of plant sciences, 93, 209, 289, 293, 419
 critical tables, 291
 Ithaca Congress, Plant physiology at, 293

J

- JOHNSTON, EARL S., Plant photometer, 89

K

- Kansas City meeting, 93
 KERR, W. E., Climatic effects in metabolism of maize, 415
 KOCH, FRED C., Sulphur metabolism of yeast, 337
 KOSTYTSCHEW, S., Chemical plant physiology, 211
 KRUYT, H. R., Einführung in die physikalische Chemie und Kolloidchemie, 421

L

- LEE, H. ATHERTON, Root distribution of sugar cane, 363
Lemna, reproduction and growth-promoting substances, 273
 LEPESCHKIN, W., Lehrbuch der Pflanzenphysiologie, 94
 Level, constant, for culture solutions, 417
 Libraries, research, 211
 Life membership, fund, 206
 Light, polarized, and starch hydrolysis, 201
 LIPMAN, C. B., Zinc and boron for green plants, 231
 Literature, citation of, 206
 LIVINGSTON, BURTON E., Growth of wheat in auto-irrigated soil, 387
 LOOMIS, W. E., Clearing plant extracts, 179
 Analysis of plant tissues, 397
 Potassium oxalate as deleading agent, 403
Lycopersicon esculentum, 3, 194, 265

M

- Maize, climatic effects in metabolism, 415

MARTIN, W. M., Plant tissue fluids, 57
 MAXIMOW, N. A., Physiological basis of
 drought resistance of plants, 213
 Mechanism, of accumulation of dyes, 215
 nervous, in plants, 291
 Meeting, Kansas City, 93
 regional, St. Paul, 289
 summer, 210
 Membership, life fund, 206
 Metabolism, sulphur, of yeast, 337
 temperature effects, in wheat, 307
 Methods, analytical, committee on, 291
 recommendations of committee, 397
 Methylated glucoses, digestion of, 379
 MILLER, E. V., Silver sols, 409
 Minnesota section, 210, 290, 420
 MURNEEK ANDREW EDWARD, Correlation
 between vegetative and reproductive
 functions, 3

N

Nervous mechanism of plants, BOSE, 291
 NEWTON, R., Plant tissue fluids, 57
 Nitrates, colorimetric estimation in fresh
 tissues, 191
 Nutrition, studies on *Fusarium lini*, 151

O

Officers, American Society of Plant Physi-
 ologists, 289
 Official and tentative methods, Associa-
 tion of Official Agricultural Chem-
 ists, 212
 Oxalate, potassium, as deleading agent,
 403
 Oxidation, of certain fatty acids, 349
 Oxygen, requisite for seed germination,
 95, 139
 supplying power of soil, 95

P

Pectin, digestion of, 379
 PIERCE, G. J., Physiology of plants, 292
 PHILLIPS, T. G., Analysis of plant tis-
 sues, 397
 Phosphates, colorimetric estimation in
 fresh tissues, 191
 Photometer, plant, 89

Photosynthesis, STILES, 94
 SPOEHR, 421
 Physical chemistry and colloid chemistry,
 KRUYT, 421
 Physikalische Chemie der Zelle und der
 Gewebe, HÖBER, 421
 Physiology of plants, PIERCE, 292
 plant, at the Ithaca congress, 293
 Plant extracts, clearing, 179
 nutrition and plant production, RUS-
 SELL, 422
 photometer, 89
 physiology at Ithaca congress, 293
 chemical, KOSTYTSCHEW, 211
 text book of, LEFESCHKIN, 94
 science, congress, 93, 209, 289, 419
 solutions, colorimetric estimations of
 nitrates, phosphates, potassium, 191
 tissues, chemical analysis of, 397
 Polarized light, and starch hydrolysis,
 201
 Potassium, colorimetric estimation in
 plant solutions, 191
 oxalate, as deleading agent, 403
 Program committee, 290
 Protoplasm, 419
 Publication, scientific, 90
 Purdue University section, 210

R

Rainwater, sulphur in, 77
 REGEIMBAL, L. O., Conductivity cell for
 continuous respiration, 205
 Regional meeting, St. Paul, 289
 Reproduction of *Lemna* and growth pro-
 moting substances, 273
 Reproductive functions, correlated with
 vegetation, 3
 Research libraries, 211
 Resistance, drought, physiological basis,
 MAXIMOW, 213
 to low temperatures, clover, 281
 Respiration, conductivity cell for con-
 tinuous study, 205
 REYNOLDS, ERNEST SHAW, Nutritional
 study of *Fusarium lini*, 151
 RHINE, J. B., Oxidation of fatty acids,
 349

Ripening, chemical changes in Grimes apple, 251

Roots, distribution in soil, sugar cane, 363

RUSSELL, SIR JOHN, Plant nutrition and plant production, 422

S

St. Paul, regional meeting, 289

SCARTH, G. W., Accumulation of dyes, 215

Scientific publication, 90

Section, Minnesota, 210, 290, 420

Purdue University, 210

Seed germination, oxygen requisite for, 139

Seeds, aqueous extracts for preparation of silver sols, 409
for investigation, 94

SEMMENS, ELIZABETH SYDNEY, Hydrolysis of starch by polarized light, 201

SHULL, C. A., Citation of literature, 206
Scientific publication, 90

Silver sols, seed extracts for preparation of, 409

Society dues, 419

Soil, oxygen supplying power, 95
auto-irrigated, growth of wheat in, 387

Sols, silver, seed extracts for preparation of, 409

Solutions, culture, constant level, 417

SOMMER, A. L., Zinc and boron for green plants, 231

SPOEHR, H. A., Photosynthesis, 421

Starch, hydrolysis by polarized light, 201

STEINBAUER, GEORGE, Resistance of clovers to low temperatures, 281

STILES, W., Photosynthesis, 94

Storage, chemical changes of Grimes apple during, 251
food, 287

Sugar cane, distribution of roots in Hawaiian soils, 363

SUGATA, H., Sulphur metabolism of yeast, 337

Sulphur, in rainwater, 77
metabolism of yeast, 337

Summer meeting, 210

Surface equilibria, Du Noüy, 421

T

Tables, international critical, 291

Temperature, effects in metabolism in wheat, 307

low, resistance of clovers to, 281

THONE, FRANK, Plant physiology at the Ithaca Congress, 293

Tissue fluids, extraction, 57

Tissues, plant, chemical analysis of, 397

Tomato, correlation of vegetative and reproductive functions, 3

growth studies, 265

nitrate nitrogen in, 194

TOTTINGHAM, W. E., Analysis of plant tissues, 397

Climatic effects in metabolism of maize, 415

Temperature effects in metabolism of wheat, 307

U

Ultraviolet rays, chemical action of, ELLIS and WELLS, 212

University of Minnesota section, 210, 290, 420

Purdue, section, 210

V

Vegetative functions, correlated with reproduction in tomato, 3

W

Water-supplying power of soil, and wheat growth, 387

WELLS, A. A., Chemical action of ultraviolet rays, 212

Wheat, growth in auto-irrigated soils, 387

temperature effects in metabolism of, 307

WILLAMAN, J. J., Analysis of plant tissues, 397

WILSON, J. DEAN, Growth of wheat in auto-irrigated soils, 387

Y

Yeast, growth of, 67
sulphur metabolism of, 337

Z

Zinc, indispensable for higher plants, 231

MEMBERSHIP LIST

- Andrews, F. M. Indiana University, Bloomington, Ind.
Bakke, A. L. Iowa State College, Ames, Iowa.
Ball, C. R. Office of Cereal Investigations, U. S. Dept. Agr., Washington,
D. C.
Becking, L. G. M. Stanford University, Palo Alto, California.
Beeskow, H. C. University of Chicago, Chicago, Ill.
Benediet, H. M. University of Cincinnati, Cincinnati, O.
Bennett, J. P. Univ. of Calif., Berkeley, Calif.
Bioletti, F. T. Univ. of Calif., Berkeley, Calif.
Bonnett, L. O. University Farm, Davis, Calif.
Bowman, H. H. M. Univ. of Toledo, Toledo, Ohio.
Brannon, J. R. Univ. of Illinois, Urbana, Ill.
Broek, J. A. Continental Sugar Co., Toledo, Ohio.
Brown, H. D. Purdue University, Lafayette, Indiana.
Brown, J. G. Univ. of Arizona, Tucson, Arizona.
Burrell, B. I. University of Minnesota, St. Paul, Minn.
Bushnell, J. W. Ohio Agr. Exp. Sta., Wooster, Ohio.
Cannon, W. A. Palo Alto, Calif.
Carr, R. H. Purdue University, Lafayette, Indiana.
Carrick, D. B. Cornell University, Ithaca, N. Y.
Clark, N. A. Iowa State College, Ames, Iowa.
Clements, F. E. Carnegie Institution, Mission Canon, Santa Barbara,
Calif.
Clements, H. F. Michigan State College, East Lansing, Mich.
Clinton, G. P. Conn. Agr. Exp. Sta., New Haven, Conn.
Cody, M. D. Univ. of Florida, Gainesville, Fla.
Cooper, D. C. Purdue University, Lafayette, Ind.
Cullinan, F. P. Purdue University, Lafayette, Ind.
Darrow, G. M. Bureau of Plant Ind., U. S. Dept. Agr., Glenn Dale, Mary-
land.
Davis, L. D. Purdue University, Lafayette, Indiana.
Davis, W. B. U. S. Dept. Agr. Lab. Fruit and Vegetable Chem., Los An-
geles, Calif.
Doubt, S. L. Washburn College, Topeka, Kansas.
Drain, B. D. Massachusetts Agr. Coll., Amherst, Mass.
Duddleston, B. H. Purdue Univ., Lafayette, Ind.
Dungan, G. H. Univ. of Illinois, Urbana, Ill.
Dunn, S. J. Iowa State College, Ames, Iowa.

PLANT PHYSIOLOGY

- Durrell, L. W. Colorado Agricultural College, Fort Collins, Col.
 Dustman, R. B. Univ. of West Va., Morgantown, West Va.
 Eaton, F. M. Escondido, Calif.
 Eaton, S. V. University of Chicago, Chicago, Ill.
 Ezekiel, W. N. Univ. of Minnesota, St. Paul, Minn.
 Fergus, E. N. University of Kentucky, Lexington, Ky.
 Fulmer, E. I. Iowa State College, Ames, Iowa.
 Gardner, W. A. Alabama Polytechnic Institute, Auburn, Ala.
 Gerhardt, F. Iowa State College, Ames, Iowa.
 Gericke, W. F. University of Calif., Berkeley, Calif.
 Gilbert, B. E. Rhode Island Exp. Sta., Kingston, Rhode Island.
 Goldsmith, G. W. Carnegie Institution, Colorado Springs, Colorado.
 Harrison, T. L. University of Manitoba, Winnipeg, Canada.
 Hart, H. University of Minnesota, St. Paul, Minn.
 Hartt, C. E. St. Lawrence Univ., Canton, N. Y.
 Harvey, E. M. Oregon Agr. Coll., Corvallis, Oregon.
 Harvey, R. B. University of Minnesota, St. Paul, Minn.
 Heath, H. C. Westminster College, Fulton, Mo.
 Hendrickson, A. H. Univ. of Calif., Davis, Calif.
 Hibbard, R. P. Michigan State Coll., East Lansing, Mich.
 Hill, G. R., Jr. Utah Agr. Coll. Logan, Utah.
 Hixon, R. M. Iowa State Coll., Ames, Iowa.
 Hoagland, D. R. Univ. of Calif., Berkeley, Calif.
 Hoffer, G. N. Purdue University, Lafayette, Indiana.
 Horr, W. H. University of Kansas, Lawrence, Kas.
 Hutchins, L. M. Bureau of Plant Industry, U. S. Peach Disease Field
 Lab., Fort Valley, Ga.
 Inman, O. L. Antioch College, Yellow Springs, Ohio.
 Ireland, J. C. S. E. State Teachers' College, Durant, Oklahoma.
 Jacobsen, H. G. M. University of Arkansas, Fayetteville, Ark.
 Jensen, O. F. National Fertilizer Co., Chicago, Ill.
 Johnston, E. S. Univ. of Maryland, College Park, Maryland.
 Johnstone, G. R. Univ. of So. Calif., Los Angeles, Calif.
 Jones, J. P. Massachusetts Agr. Coll., Amherst, Mass.
 Keger, A. Colorado Agr. Coll., Fort Collins, Colorado.
 Korstian, C. F. Yale School of Forestry, New Haven, Conn.
 Larson, A. H. Univ. of Minnesota, St. Paul, Minn.
 Lehenbauer, P. A. University of Nevada, Reno, Nevada.
 Lill, J. G. Michigan State College, East Lansing, Mich.
 Lincoln, F. B. Univ. of Calif., Berkeley, Calif.
 Lipman, C. B. Univ. of Calif., Berkeley, Calif.

MEMBERS

Livingston, B. E. Johns Hopkins Univ., Baltimore, Md.
Lloyd, F. E. McGill University, Montreal, Canada.
Loehwing, W. F. Univ. of Iowa, Iowa City, Iowa.
Loomis, W. E. Univ. of Arkansas, Fayetteville, Ark.
McAllister, F. University of Texas, Austin, Texas.
McGillivray, J. H. Purdue Univ., Lafayette, Indiana.
McGinnies, W. G. University of Arizona, Tucson, Arizona.
McHargue, J. S. University of Kentucky, Lexington, Ky.
MacMillan, H. G. Bureau Plant Ind., Greeley, Colorado.
Mackie, W. W. Univ. of Calif., Berkeley, Calif.
Madson, B. A. Univ. Farm, Davis, Calif.
Mains, E. B. Purdue University, Lafayette, Indiana.
Marsh, R. P. Gettysburg College, Gettysburg, Pa.
Massey, L. M. Cornell Univ., Ithaca, N. Y.
Milad, Y. Univ. of Calif., Berkeley, Calif.
Moore, C. S. Univ. of Redlands, Redlands, Calif.
Moore, D. M. Univ. of Arkansas, Fayetteville, Arkansas.
Murneek, A. E. Univ. of Missouri, Columbia, Mo.
Newton, R. Univ. of Alberta, Edmonton, Canada.
Palmer, M. Alabama Polytechnic Institute, Auburn, Ala.
Partridge, N. L. Michigan State College, East Lansing, Mich.
Petry, E. J. Univ. of So. Dakota, Brookings, So. Dak.
Pessin, L. J. Texas Agr. Coll., College Station, Texas.
Pittman, D. W. Utah Agr. College, Logan, Utah.
Porter, C. L. Purdue University, Lafayette, Indiana.
Quinn, J. T. Univ. of Missouri, Columbia, Mo.
Raleigh, G. J. University of Chicago, Chicago, Ill.
Remberta, Sister. St. Benedict's Academy, St. Joseph, Minn.
Reynolds, E. S. Agricultural College, No. Dakota.
Rhine, J. B. University of West Va., Morgantown, West Va.
Richey, H. W. Iowa State College, Ames, Iowa.
Rogers, C. F. Colorado Agr. College, Fort Collins, Colorado.
Rose, J. P. Ashland, Oregon.
Rumbold, C. T. University of Wisconsin, Madison, Wis.
Saeger, A. Junior College of Kansas City, Kas. City, Mo.
St. John, R. R. Purdue University, Lafayette, Indiana.
Schley, E. O. 205 No. Mayfield Ave., Chicago, Ill.
Schoonover, W. P. Univ. of Calif., Berkeley, Calif.
Schultz, O. C. Oklahoma Agr. Coll., Stillwater, Oklahoma.
Setchell, W. A. Univ. of Calif., Berkeley, Calif.
Severance, H. M. Univ. of So. Calif., Los Angeles, Calif.

PLANT PHYSIOLOGY

- Severy, J. W. Montana State University, Missoula, Montana.
Shirk, C. J. Nebraska Wesleyan Univ., University Place, Neb.
Shull, C. A. Univ. of Chicago, Chicago, Ill.
Shull, G. H. Princeton Univ., Princeton, N. J.
Smith, G. M. Purdue University, Lafayette, Indiana.
Smith, O. Iowa State College, Ames, Iowa.
Snyder, R. M. Michigan State College, East Lansing, Michigan.
Sommer, A. L. Oakland, Calif.
Steinbauer, P. G. University of Minnesota, St. Paul, Minn.
Steinmetz, F. H. Univ. of Minnesota, St. Paul, Minn.
Swartout, H. G. Univ. of Missouri, Columbia, Mo.
Thone, F. Science Service, Washington, D. C.
Tottingham, W. E. University of Wisconsin, Madison, Wis.
Traub, H. P. Univ. of Minnesota, St. Paul, Minn.
Trelease, S. F. Columbia University, New York.
Trost, J. F. Purdue University, Lafayette, Indiana.
Wakabayashi, S. 216 Fifth Ave. S., Seattle, Washington.
Williams, A. R. 6322 Drexel Ave., Chicago, Ill.
Wolfe, H. S. University of Chicago, Chicago, Ill.
Young, V. A. University of Minnesota, Minneapolis, Minn.

Indian Agricultural Research Institute (Pusa)
LIBRARY, NEW DELHI-110012

This book can be issued on or before

Return Date	Return Date